

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
25 October 2001 (25.10.2001)

PCT

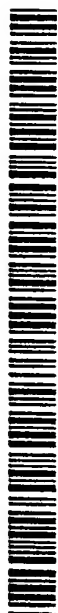
(10) International Publication Number
WO 01/78796 A1

- (51) International Patent Classification⁷: **A61L 9/04**
- (21) International Application Number: **PCT/GB01/01755**
- (22) International Filing Date: **17 April 2001 (17.04.2001)**
- (25) Filing Language: **English**
- (26) Publication Language: **English**
- (30) Priority Data:
- | | | |
|------------|----------------------------|----|
| 0009269.2 | 15 April 2000 (15.04.2000) | GB |
| 0014761.2 | 17 June 2000 (17.06.2000) | GB |
| 60/215,232 | 30 June 2000 (30.06.2000) | US |
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- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- Published:**
- with international search report
 - before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: **TYPE II GONADOTROPIN-RELEASING HORMONE RECEPTOR AND POLYNUCLEOTIDES ENCODING THEREFOR**

(57) Abstract: There is provided polynucleotides encoding the full sequence for the marmoset and human Type II gonadotropin-releasing hormone receptors (Type II GnRH-R). The corresponding amino acid sequences are also provided.

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1 **"Type II Gonadotropin-releasing Hormone Receptor**
2 **and Polynucleotides Encoding Therefor"**

3

4 The present invention relates to a novel Type II
5 gonadotropin-releasing hormone receptor (Type II
6 GnRH-R), to genetically engineered host cells able
7 to express the Type II GnRH-R, and the ligands and
8 antibodies therefor.

9

10 Type I gonadotropin-releasing hormone (GnRH) is a
11 decapeptide released from the hypothalamus, and
12 acts through receptors to regulate the secretion of
13 gonadotropins required for reproductive function
14 (see Fink et al., "Gonadotrophin secretion and its
15 control", The Physiology of Reproduction, E Knobil
16 and I Neill, New York, Raven Press, pages 1349-
17 1377, 1988).

18

19 Receptors for Type I GnRH (ie Type I GnRH-R) are
20 members of the large G-protein-coupled receptor
21 family and are preferentially coupled to

1 phosphoinositidase C via the G_q/G_{11} family of G
2 proteins. Typically Type I GnRH-Rs are located in
3 the gonadotroph cells of the anterior pituitary
4 gland (where binding of Type I GnRH leads to
5 release of the gonadotropins luteinising hormone
6 and follicle-stimulating hormone), as well as on
7 the central and peripheral nervous systems, gonads,
8 placenta and on certain tumours, such as breast and
9 prostate. Type I GnRH receptors may display both
10 up and down regulation and Type I GnRH agonists
11 have been used in management of prostate and breast
12 cancer, as well as to stimulate gonadotropin
13 secretion in the treatment of infertility.

14
15 Expression of mouse and rat Type I GnRH-R was first
16 achieved by injecting poly(A)⁺ mRNA from a suitable
17 source (eg from the pituitary gland) into *Xenopus*
18 oocytes (see, for example, Eidne et al., J. Mol.
19 Endocr. Vol 1, pages R9-R12, 1988; Yoshida et al.,
20 Molecular Endocrinology, Vol 3, pages 1953-1960,
21 1989; and Sealfon et al., Molecular Endocrinology,
22 Vol 4, pages 119-124, 1990). This system allowed
23 some characterisation of the pharmacology of the
24 Type I GnRH-R.

25
26 The protein-encoding nucleotide sequence of the
27 murine Type I GnRH-R was first published by
28 Tsutsumi et al., (Molecular Endocrinology, Vol 6,
29 pages 1163-1169, 1992) together with the deduced
30 amino acid sequence for murine Type I GnRH-R.

1 Eleven different forms of GnRH in vertebrates have
2 been identified to date (see King and Millar, "Co-
3 ordinated evolution of GnRHs and their receptors",
4 in GnRH Neurons: Gene to Behavior, Eds. I.S. Parhar
5 and Y. Sakuma, Brain Shuppan, Tokyo, pages 51-77,
6 1997; see Sealfon et al., Endocr. Rev. 18:180-205,
7 1997; Millar et al., "Plasticity in the structural
8 and functional evolution of GnRH: A peptide for all
9 seasons", in Proceedings of the XIIIth
10 International Conference of Comparative
11 Endocrinology, Eds. S. Kawashima and S. Kikuyama,
12 Moduzzi Editore, Italy, pages 15-27, 1997; and see
13 Sherwood et al., General and Comparative
14 Endocrinology, 112, 1998). Many of these different
15 GnRH forms are in fact variants of GnRH Type I.
16 However, distinct Type II and Type III GnRHs have
17 been identified.

18
19 Type II GnRH was originally isolated from chicken
20 brain (see Millar and King, News Physiological
21 Science, 3:49-53, 1988) and was initially termed
22 "chicken GnRH II" or "cGnRH II". Subsequent
23 investigations have revealed that this isoform is
24 present in most vertebrate species, and of all the
25 GnRH isoforms GnRH II is the most ubiquitous. The
26 wide distribution of GnRH II suggests an important
27 function and it is postulated to have a
28 neuromodulatory, and possibly a neuroendocrine,
29 role in the central and peripheral nervous systems
30 (see Millar and King, 1988, supra). GnRH II has
31 been shown to regulate M currents (K^+ channels) in

1 the sympathetic ganglion (Bosma et al., in G
2 proteins and Signal Transduction, The Rockefeller
3 University Press, pages 43-59, 1990) and stimulates
4 reproductive behaviour (see King et al., in GnRH
5 Neurones : Gene to Behavior, eds. Parhar (Brain
6 Shuppan), Tokyo, pages 51-77, 1997. It has also
7 been postulated that GnRH II acts as a specific
8 FSH-releasing agent (Millar et al. Ref No. 33).

9
10 Type II GnRH is highly expressed in kidney, bone
11 marrow and prostate tissues as well as the
12 extrahypothalamic brain (White et al. Ref No. 15).

13
14 To date only partial sequence information has been
15 available for a Type II GnRH-R. Specifically,
16 Millar et al. (in Journal of Endocrinology,
17 162:117-126, 1999) reported a continuous nucleotide
18 sequence of 1642 nucleotides of the human gene,
19 obtained by screening the human genome EST
20 (expressed sequence tag) database. The EST
21 sequences were confirmed in the cloned human gene
22 and in PCR products of cDNA from several tissues.
23 All the EST transcripts detected were in the
24 antisense orientation with respect to the novel
25 GnRH receptor sequences herein described and were
26 highly expressed in a wide range of human brain and
27 peripheral tissues.

28

29 PCR analysis of the cDNA partial sequence obtained
30 by Millar et al., revealed that an intronic
31 sequence equivalent to intron 2 of human Type I

1 GnRH-R was retained. The intron itself was not
2 spliced out in the transcript, but this was
3 expected for anti-sense transcripts, as candidate
4 donor and acceptor sites were only present in the
5 gene when transcribed in the orientation encoding
6 the GnRH receptor homolog. Despite extensive 5'
7 RACE studies Millar et al. did not obtain any
8 transcript 5' to the sequence corresponding to
9 intron 2 of human Type I GnRH-R, and the antisense
10 transcripts terminated in poly A due to the
11 presence of a polyadenylation signal sequence in
12 the putative intron 2 when transcribed in the
13 antisense orientation (see Fig. 1).

14
15 None of the sequence revealed any contiguous open-
16 reading frame which would translate a functional
17 protein. Millar et al. concluded that the putative
18 receptor was probably a pseudogene representing a
19 receptor which had become redundant and further
20 investigations have revealed that the full-length
21 antisense transcript encodes a novel
22 ribonucleoprotein (RNP) which localised to
23 chromosome 14 (Ref 26). Subsequently it was found
24 that the gene was a pseudogene for both RNP and
25 Type II GnRH-R.

26
27 The presence of the RNP explains the widespread
28 tissue expression observed and it is significant
29 that only the 3' untranslated sequences of the RNP
30 cDNA overlap the putative GnRH Type II receptor

1 sequences encoding the equivalent of exon 1 and
2 exon 2.

3

4 Despite the findings of Millar et al. which suggest
5 that an operative version of GnRH-R Type II does
6 not exist in mammals, we have now obtained the full
7 nucleotide sequence encoding the Type II GnRH-R
8 from marmoset and also the complete nucleotide
9 sequence (including exon I) encoding the human Type
10 II GnRH-R. The human Type II GnRH-R herein
11 reported has been localised to chromosome 1 (1q12-
12 21).

13

14 The present invention thus provides a
15 polynucleotide encoding a functional Type II
16 gonadotropin-releasing hormone receptor (Type II
17 GnRH-R) peptide.

18

19 The term "peptide" is used herein to refer to any
20 peptidal compound without connotation of size, and
21 includes therefore larger molecules which elsewhere
22 may alternatively be termed "polypeptides" or
23 "proteins" also fall within this definition.

24

25 In one embodiment the peptide encoded comprises at
26 least a portion of exon I. By "exon I" we refer to
27 that portion of Type II GnRH-R which corresponds to
28 and exhibits substantial homology with exon I of
29 Type I GnRH-R. In particular we refer to a peptide
30 which includes over 90% of the amino acid sequence
31 1 to 170 in the marmoset Type II GnRH-R sequence of

1 SEQ ID No 2 or to a peptide which includes the
2 equivalent amino acids of the human Type II GnRH-R
3 sequence. Thus, for example, exon I refers to
4 amino acid nos. 1 to 168 of splicing alternative 1
5 as set out in SEQ ID No 5 (see Fig. 3). The above
6 terminology has been adopted for clarity; the short
7 splice referred to below is herein designated
8 intron 1' to distinguish it from intron 1.
9 Consequently the short exon (exon 1') comprising
10 amino acids nos. 1 to 9 of the human Type II GnRH-R
11 peptide is incorporated into exon I as herein
12 defined.

13

14 Preferred embodiments of the present invention
15 include polynucleotides having a nucleotide
16 sequence as set out in SEQ ID No 1 (marmoset) or
17 SEQ ID No 3 (human) or polynucleotides which encode
18 a polypeptide having an amino acid sequence as set
19 out in SEQ ID No 2 (marmoset) or SEQ ID Nos 4, 6, 8
20 or 10 (human).

21

22 As shown in Fig. 3 the marmoset amino acid sequence
23 of Type II GnRH-R has over 90% homology with the
24 corresponding human sequence. Accordingly, the
25 present invention incorporates any polypeptide
26 having at least 90% homology with the amino acid
27 sequence of SEQ ID Nos. 1 or 3.

28

29 When the human nucleotide sequence is compared to
30 that of the marmoset, there is an apparent single-
31 base deletion at position 30 of the coding

1 sequence. The earlier EST sequence (Genbank
 2 BG036291) matches the 5' end of the sequence, but
 3 the match ends at nucleotide 29 of the coding
 4 sequence and continues from nucleotide 290 of the
 5 coding sequence, indicating that this is a splice
 6 site. In the chicken receptor an intron was also
 7 located in a similar position. Excision of a very
 8 short intron (intron 1') at this position would
 9 account for the frame shift; very short introns
 10 have been noted in other genes, for example the
 11 mouse alpha-7 integrin gene has an intron of only
 12 16 bases (Genbank L23422). There are three
 13 alternative splicing possibilities, involving 5-,
 14 8- or 38-base deletions, each of which would
 15 restore the open reading frame of the human Type II
 16 GnRH-R.

17
 18 The start of the human Type II GnRH-R amino acid
 19 sequence following each of the three splicing
 20 alternatives is set out below:

21

22 **Splicing alternative 1:**

23 (5 bases deleted from position 29 on)

24

25 Met Ser Ala Gly Asn Gly Thr Pro Trp 9

26 1 ATG TCT GCA GGC AAC GGC ACC CCT TGG

27

28

29 Ala Ala Gly Glu Glu Val Trp Ala 17

30 28 GCA GCG GGG GAG GAG GTC TGG GCT

31

1 Splicing alternative 2:

2 (8 bases deleted from position 29 on)

3

4 Met Ser Ala Gly Asn Gly Thr Pro Trp 9

5 1 ATG TCT GCA GGC AAC GGC ACC CCT TGG

6

7

8 Ala Gly Glu Glu Val Trp Ala Gly 17

9 28 GCG GGG GAG GAG GTC TGG GCT GGA

10

11 Splicing alternative 3:

12 (38 bases deleted from position 29 on)

13

14 Met Ser Ala Gly Asn Gly Thr Pro Trp 9

15 1 ATG TCT GCA GGC AAC GGC ACC CCT TGG

16

17 Val Glu Val Glu Gly Ser Glu Leu 17

18 28 GTG GAG GTG GAG GGC TCA GAG CTG

19

20

21 The full sequences for human Type II GnRH-R
22 according to the first splicing alternative are set
23 out in SEQ ID Nos 5 and 6, according to the second
24 splicing alternative in SEQ ID Nos 7 and 8, and
25 according to the third splicing alternative in SEQ
26 ID Nos 9 and 10.

27

28 As shown in SEQ ID No. 3, the human nucleotide
29 sequence includes an apparent stop codon in the
30 first part of exon 2. In the marmoset the
31 equivalent codon (shown at nucleotides 449-452 of

1 SEQ ID No 1) represents an arginine. A modified
2 version of SEQ ID No 3 whereby the stop codon is
3 engineered to represent an amino acid (for example
4 arginine) is hereby incorporated. However, we
5 believe that the codon TGA does not function as
6 stop codon, but is instead translated as
7 selenocysteine. In eukaryotes decoding of TGA as
8 selenocysteine requires the selenocysteine
9 insertion element (SECIS) in the 3' UTR of the mRNA
10 (see Copeland et al., EMBO J, 19(2): 306-14, 2000;
11 and Fagegaltier et al., Nucleic Acids Research,
12 29(14): 2679-80, 1995). The putative UTR of the
13 sequence (see SEQ ID No. 3) was found to contain
14 the SECIS pattern
15 RTGAN{13,15}AARN{23,26}GA. Thus, selenocysteine
16 insertion at that position of the protein (amino
17 acid no 177 in SEQ ID No 6; amino acid no 176 in
18 SEQ ID No 8; amino acid no 166 in SEQ ID No 10) is
19 possible. In SEQ ID Nos. 3, 5, 7 and 9 the TGA in
20 shown as Xaa (and defined as TGA in the preamble to
21 the sequence) simply to maintain the coding
22 sequence as a whole in the PATENTIN program.
23
24 Desirably the polynucleotide according to the
25 present invention encodes a peptide which is able
26 to bind specifically to Type II GnRH and,
27 preferably, is able to function as a receptor
28 therefor.
29
30 Those skilled in the art will appreciate that the
31 nucleotide sequences of SEQ ID No 1 and Nos 3, 5, 7

1 and 9 correspond to one allele of the marmoset and
2 human gene respectively, and that allelic variation
3 is likely. Allelic variants can be cloned by
4 probing cDNA or genomic libraries from different
5 individuals according to standard procedures.
6 Allelic variants of the DNA sequences shown in the
7 sequence listing, including those containing silent
8 mutations and those in which mutations result in
9 amino acid sequence changes, are within the scope
10 of the present invention, as are proteins which are
11 allelic variants of SEQ ID No 2 and Nos 4, 6, 8 and
12 10.

13
14 The present invention also comprises
15 polynucleotides encoding homologous Type II GnRH-Rs
16 from other species, preferably other mammalian
17 species. Murine, porcine, ovine, bovine, canine,
18 feline, equine and primate Type II GnRH-Rs are of
19 particular interest. The sequence information
20 provided in SEQ ID Nos 1, 3, 5, 7 and 9 together
21 with the techniques described herein and the
22 standard conventional cloning techniques known in
23 the art are sufficient to obtain such homologous
24 polynucleotides. For example, there is a
25 substantial body of knowledge concerning the
26 techniques required for the art of genetic
27 engineering and reference is made to Maniatis et
28 al, Molecular Cloning, A Laboratory Manual, Cold
29 Spring Harbor Laboratory, Cold Spring Harbor, New
30 York 1982 and "Principles of Genetic Engineering",
31 Old and Primrose, fifth addition, 1994.

1 The present invention also includes modified
2 sequences retaining Type II GnRH-R function (i.e.
3 the ability to bind GnRH Type II) and having at
4 least 70% homology (preferably 80% homology,
5 especially preferably 85-90% homology and most
6 preferably over 90% homology) with the nucleotide
7 sequence in question. Functional equivalents of
8 such polynucleotides are also part of this
9 invention. In particular, we include nucleotide
10 substitutions which do not affect the amino acid
11 expressed. The term "functional equivalent" used
12 herein refers to any derivative in which
13 nucleotide(s) and/or amino acid(s) have been added,
14 deleted or replaced without a significantly adverse
15 effect on expression of the gene product or on
16 biological function thereof. Thus, for example,
17 amino acid Glu may be encoded by the codon gag or
18 by the codon gaa and each construct may be varied
19 in this way without affecting the sequence of the
20 expressed peptide. Additionally, most vertebrates
21 have three types of GnRH and it is postulated that
22 three cognate receptors exist. Together with
23 collaborators (Troskie, Kwon and Wangli) we have
24 identified Types I, II and III GnRH receptors in
25 Xenopus and bullfrog. The Type III GnRH-R is more
26 similar to Type II GnRH-R than Type I. We
27 therefore propose the existence of a Type III human
28 GnRH-R with homology to Type II. Thus, the Type
29 III GnRH-R is expected to have substantial homology
30 with the Type II GnRH-R described herein and hence

1 will be covered by the modified versions of the
2 sequences disclosed.

3
4 The polynucleotides may be in any form (for example
5 DNA or RNA, double or single stranded) but
6 generally double stranded DNA is the most
7 convenient. Likewise the polynucleotides according
8 to the present invention may be part of a
9 recombinant genetic construct, which itself may
10 include a vector (for example an expression vector)
11 and eukaryotic vectors (as well as prokaryotic
12 vectors) are of interest. Alternatively, the
13 construct may be incorporated into the genome of a
14 transgenic animal. Any vectors or transgenic
15 animals comprising a polynucleotide as described
16 above form a further aspect of the present
17 invention.

18
19 Viewed in a yet further aspect the present
20 invention provides a recombinant expression system
21 able to express the Type II GnRH-R described above.
22 DNA constructs (i.e. a standard vector
23 recombinantly combined with a polynucleotide
24 sequence coding for the Type II GnRH-R of interest)
25 and cells transformed with such constructs are also
26 encompassed by the present invention.

27
28 The term "expression system" is used herein to
29 refer to a genetic sequence which includes a
30 protein-encoding region and is operably linked to
31 all of the genetic signals necessary to achieve

1 expression of that region. Optionally, the
2 expression system may also include a regulatory
3 element, such as a promoter or enhancer, to
4 increase transcription and/or translation of the
5 protein encoding region or to provide a control
6 over expression. The regulatory element may be
7 located upstream or downstream of the protein
8 encoding region or within the protein encoding
9 region itself.

10

11 In addition to the Type II GnRH-R construct
12 described above, the present invention also
13 provides host cells transformed with such
14 constructs and which may express the biologically
15 active modified gene product.

16

17 In a further aspect, the present invention provides
18 a stable cell-line capable of expressing Type II
19 GnRH-R, preferably human Type II GnRH-R, as
20 described above. By "stable" we mean that the
21 cell-line retains its ability to express useful
22 quantities of Type II GnRH-R after several (e.g.
23 10) generations, with any decrease in the level of
24 Type II GnRH-R expression being sufficiently low
25 not to materially affect the utility of the cell-
26 line.

27

28 Desirably the host cell transformed with the
29 construct encoding the human Type II GnRH-R is of
30 mammalian origin, but other cell types may also be
31 useful. Examples include prokaryotic cells (such

1 as *E. coli*), non-mammalian derived eukaryotic cells
2 (such as insect, yeast or plant cells). Suitable
3 host cells include, for example, COS-1 cells, COS-7
4 cells, COSM6 cells, CHO cells, BHK cells, GH₃
5 cells, HEK293 cells and 293EBNA cells.

6
7 The present invention also provides isolated
8 peptides comprising a functional Type II
9 gonadotropin-releasing hormone receptor (Type II
10 GnRH-R) peptide. Preferably said peptide comprises
11 at least a portion of exon I.

12
13 Preferred embodiments of the peptides according to
14 the present invention include peptides having an
15 amino acid sequence corresponding to the sequence
16 of amino acid nos 1 to 170 of SEQ ID No 2
17 (preferably comprising substantially the full
18 sequence as set out in SEQ ID No 2) or
19 corresponding to the sequence of amino acid nos 1
20 to 168 of SEQ ID No 6, amino acid nos 1 to 165 of
21 SEQ ID No 8 or amino acid nos 1 to 155 of SEQ ID No
22 10 (preferably also comprising substantially the
23 full sequence of SEQ ID Nos 4, 6, 8 or 10).

24

25 Desirably, the peptide is able to bind to Type II
26 GnRH and, preferably, is a functional receptor
27 therefor.

28

29 The present invention also provides antibodies
30 specific to Type II GnRH-R peptides, preferably
31 antibodies which are specific to the extracellular

1 domains of Type II GnRH-R, for example EC3 in exon
2 3 thereof. The term "antibodies" as used herein
3 includes not only monoclonal and polyclonal
4 antibodies, but also antigen binding fragments
5 thereof, trimeric or tetrameric constructs and
6 recombinant or proteolytic antibody fragments.

7
8 Antibodies directed to EC1, EC2 and EC3 (especially
9 EC2) are of particular interest and may be of
10 therapeutic value for cancer treatment.

11 The Type II GnRH-R can be expressed and used to
12 screen agents of potential therapeutic interest.

13
14 Thus, the present invention further provides a
15 method of screening an agent for pharmacological
16 activity (i.e. to ascertain its utility in binding
17 to Type II GnRH-R), said method comprising:

- 18 a) providing a Type II GnRH-R peptide as
19 described above and exposing said Type II
20 GnRH-R peptide to the agent to be tested; and
21
22 b) ascertaining whether said agent interacts with
23 (for example binds specifically to) said Type
24 II GnRH-R peptide.

25
26 Labelled (eg. radio-labelled or fluorescent-
27 labelled) antibodies may be used to determine
28 whether the agent and Type II GnRH-R interact
29 together. Optionally a washing step may be
30 included to remove labels not bound to the agent or
31 GnRH-R.

1
2 An expression system able to produce the Type II
3 GnRH-R described above may be used in this method;
4 preferably the expression system will be a
5 transformed cell-line, the host cell usually being
6 of mammalian origin. Alternatively the expression
7 system may be a transgenic animal.

8
9 Optionally, the receptor expressed may be mutated
10 to produce constitutively active receptors which
11 can be used to screen for antagonists and agonists.

12
13 An expression system able to produce the Type II
14 GnRH-R described above may be used to screen agents
15 of potential therapeutic use (such as Type II GnRH
16 agonists or antagonists). Desirably, therefore the
17 expression system will imitate at least some
18 aspects of Type II GnRH-induced signal
19 transduction. Optionally, the expression system
20 may be a stable cell line, the host cell usually
21 being of mammalian origin. Alternatively the
22 expression system may be a transgenic animal.

23
24 In another embodiment of the invention, the Type II
25 GnRH-R itself or extracellular domain thereof (e.g.
26 the EC2 or EC3 loop) could be administered *in vivo*.
27 The free GnRH-R or extracellular domains (which
28 could be synthetic peptides) could competitively
29 bind to GnRH and inhibit its reaction with the
30 native receptor *in vivo*.

31

1 Alternatively, the Type II GnRH-R or an
2 extracellular domain thereof may be used as a means
3 of contraception. For example, a patient may be
4 immunised by injection with the Type II GnRH-R.
5 This will induce antibody production to the Type II
6 GnRH-R and the antibodies so produced will also
7 interact with native Type II GnRH-R affecting
8 reproductive ability. Alternatively, the exogenous
9 Type II GnRhH-R may bind competitively for
10 endogenous GnRH.

11
12 The present invention will now be further described
13 with reference to the following, non-limiting,
14 examples and Figures in which:

15
16 Fig. 1 is a schematic representation of the human
17 gene structure of Type I GnRH-R and of Type II
18 GnRH-R. The positions of the extracellular (EC)
19 and intracellular (IC) loop domains, transmembrane
20 (dark blocks) and carboxy terminal tail (C) are
21 indicated. The approximate positions of the "short
22 splice" (SS) for intron 1' and selenocysteine
23 (SeCys) in the human receptor is shown.

24
25 Fig. 2 shows the immunolocalisation of Type II GnRH
26 receptor and Luteinizing Hormone (LH) to sheep
27 pituitary:

28
29 a) immunolocalisation of Type II GnRH-R using
30 antibody against EC3;

31

- 1 b) immunolocalisation of LH to pituitary
2 gonadotropes;
3
4 c) co-localisation using antibodies a) and b) and
5 enzymatic detection;
6
7 d) co-localisation of Type II GnRH-R (black) and
8 LH (grey) by confocal microscopy; some cells
9 contain LH only but most expressing Type II
10 GnRH-R are LH positive.

11
12 Fig. 3 is a comparison of the amino acid sequences
13 of human Type II GnRH-R according to splicing
14 alternative 1, and marmoset Type II GnRH-R.

15
16 The numbering used is that of the human sequence.
17 Asterisks (*) indicate identity, and vertical
18 slashes (|) conserved substitution. Regions
19 predicted to be helical from homology modelling
20 with the rhodopsin crystal structure are indicated.
21 Exon boundaries, the position of the short splice
22 (intron 1') and the apparent stop codon in the
23 human sequence are also shown.

24

25 Fig. 4 shows the heterologous competitor binding of
26 ^{125}I -[His⁵, D-Tyr⁶]GnRH mediated by the Marmoset
27 Type II GnRH-R expressed in COS-7 cells in the
28 presence of increasing doses of the peptides (IC₅₀
29 values indicated in nM): mGnRH (mammalian GnRH, ■,
30 51.7± 1.9nM; GnRH II (GnRH Type II, ▲, 1.3±0.2nM);

1 and sGnRH (salmon GnRH, ▼, $9.8 \pm 1.4 \text{ nM}$). Error bars
2 represent s.e.m. ($n=2$).

3
4 Fig. 5 shows the total inositol phosphate
5 production mediated by the Marmoset Type II GnRH-R
6 expressed in COS-7 cells in response to
7 concentration of various peptides (EC_{50} values
8 indicated in nM) mGnRH (mammalian GnRH, ■, $33.2 \pm$
9 6.3 nM); GnRH II (GnRH Type II, ▲, $0.46 \pm 0.12 \text{ nM}$);
10 sGnRH (salmon GnRH, ▼, $8.7 \pm 0.5 \text{ nM}$); [D-Arg⁶] GnRH II
11 ([D-Arg⁶]GnRH Type II, ◆, $1.1 \pm 0.23 \text{ nM}$); and Ant 135-
12 18 (Antagonist 135-18, ●, $223 \pm 7 \text{ nM}$). Error bars
13 represent s.e.m. ($n=2$).

14
15 Fig. 6 shows receptor binding (a) and inositol
16 phosphate production (b) of mammalian GnRH I (○)
17 and GnRH II (●) in COS-7 cells transfected with
18 marmoset Type II receptor (left panel) and human
19 Type I receptor (right panel). Stimulation of
20 inositol phosphate by Type I receptor Antagonist
21 135-18 (□) at the Type II receptor is also shown.
22 Error bars represent s.e.m. of 3-6 separate
23 experiments.

24
25 Fig. 7 shows the Luteinizing hormone (LH) and
26 Follicle Stimulating Hormone (FSH) response to GnRH
27 I and II in sheep.

28 Fig. 8 shows activation of ERK2 and p38 α MAP
29 kinases by Type I (open bars) and Type II (closed
30 bars) GnRH receptors in COS-7 cells. Stimulation of

1 Type I (a) and II (b) GnRH receptors with mammalian
2 GnRH I, GnRH II and Antagonist 135-18 of
3 immunoprecipitated myc-ERK2. Inset panels depict
4 anti-phospho-ERK2 immunoblotting of anti-myc COS-7
5 cell immunoprecipitates. c, Left panel, selective
6 and time-dependent activation of p38 α by GnRH II
7 stimulation (100 nM) of Type II GnRH receptor.
8 Stimulation of Type I GnRH receptor (100 nM) failed
9 to activate p38 α MAP kinase. The right panel
10 demonstrates that both Type I and Type II GnRH
11 receptor stimulation induces a time-dependent
12 activation of ERK but that the GnRH II stimulation
13 of the Type II receptor is more prolonged. Data
14 represent mean \pm s.e.m., $n \geq 3$.

15
16 Fig. 9 shows expression of Type II GnRH receptor in
17 marmoset and human tissues. (a) RT-PCR was carried
18 out with specific primers on cDNA prepared from
19 marmoset RNA isolated from various tissues. PCR
20 products were fractionated by size on agarose gels.
21 Type II GnRH receptor levels were normalised to
22 actin RNA and represented as the log of the RNA
23 expression relative to pituitary. Hatched bars
24 indicate marmoset brain tissues, solid bars
25 indicate marmoset reproductive tissues while open
26 bars indicate other marmoset tissues. (b, c)
27 Expression of the Type II GnRH receptor in human
28 tissues was examined in Northern blots of mRNA
29 (Clontech) by hybridisation with ^{32}P labelled human
30 exon 1; (b) mRNA from human cerebellum (1),
31 cerebral cortex (2), medulla (3), spinal cord (4),

1 occipital pole (5), frontal lobe (6), temporal lobe
2 (7) and putamen (8); (c) mRNA from heart (1), whole
3 brain (2), placenta (3), lung (4), liver (5),
4 skeletal muscle (6), kidney (7) and pancreas (8).
5 Another blot showed moderate expression in the
6 amygdala and low expression in caudate nucleus,
7 corpus callosum, hippocampus, substantia nigra,
8 subthalamic nucleus and thalamus (data not shown).

9

10 Examples

11

12 Following numerous failed attempts to locate exon I
13 from the sequence reported by Millar et al.
14 (Journal of Endocrinology (1999), Vol 162, pages
15 117-126) subsequent work demonstrated that the
16 antisense sequence of Millar et al. was the 3'
17 untranslated end of an RNP which localised to
18 chromosome 14.

19

20 It was recognised that as long as exon 2 or exon 3
21 sequence information was used in PCR we would
22 always detect the 3' untranslated end of the RNP
23 since this is highly expressed in all tissues. In
24 addition, the putative intron 2 of Type II receptor
25 would always be retained. Since this transcript
26 ends with a polyadenylation in the region of the
27 equivalent of intron 1 of the putative Type II
28 receptor (read in the opposite direction to RNP),
29 we would never obtain information on exon 1 of the
30 Type II receptor.

31

1 We concluded that three new elements were necessary
2 to find the putative Type II GnRH receptor.

3
4 a) Search the data bases for a nucleotide
5 sequence encoding part of exon 1.

6
7 b) Generate an antibody against a highly specific
8 domain which is responsible for ligand
9 selectivity (see Sealfon et al., Endocr.
10 Review 18:180-205 and Troskie et al., General
11 and Comparative Endocrinology 112:296-302
12 (1998)) in extracellular loop 3 of the human
13 Type II vis-à-vis Type I receptor to determine
14 in which tissue this rare receptor is
15 expressed.

16
17 c) Undertake chromosomal localisation to
18 determine if the coding sequence exists in a
19 pseudogene locale (with RNP) and in another
20 "real" gene location elsewhere.

21

22 **Materials and Methods**

23

24 **Cloning of the marmoset Type II GnRH receptor.** RNA
25 was isolated from marmoset pituitary and brain stem
26 using RNAsol B (Biogenesis). 2 µg of RNA was
27 incubated with 1 mM dNTPs, 2 µM random hexa-
28 polynucleotides (Promega), gene specific primers or
29 anchored oligo-dT primers at 80°C for 10 min. 1x
30 RT buffer (Sigma), 1 U/µl RNAsin (Promega) and 0.5
31 U/µl AMV reverse transcriptase (Sigma) were added

1 in a total volume of 20 μ l and incubated at 55°C
2 for 2 h, then 65°C for 10 min. Primers designed to
3 the Type II marmoset GnRH receptor exon sequences.
4 Primers were chosen to span putative introns, to
5 enable detection of processed RNA in the presence
6 of possible genomic DNA contamination and the RNP
7 antisense transcript. 50 ng of purified (Qiagen)
8 cDNA produced with random hexa-polynucleotides were
9 subjected to PCR using human sequences previously
10 described (17) and human genomic sequence encoding
11 exon 1 (Zymogenetics AL 160282, BG 636291, AA
12 954764). Round 1 PCR: 5 cycles at 65°C, 23 cycles
13 at 63°C using primers S1 and A1 (S1,
14 GATGCCACCTGGAATATCACTG (SEQ ID No 17); A1,
15 AGGCAGCAGAAGG (SEQ ID No 18). Round 2 PCR: 5
16 cycles at 63°C, 25 cycles at 61°C using 1 μ l of
17 products from Round 1 as template and primers S2
18 and A2 (S2, CAGCCTGGGGACTTAGTTTCCTG (SEQ ID No 19);
19 A2,GGTTATAGGTGGTCTCTTGC (SEQ ID No 20). Products
20 were size-purified (Qiagen), cloned into pGEM-T
21 (Promega) and sequenced. Sense and antisense
22 oligonucleotides were designed from the novel
23 marmoset sequences and used in 3' and 5' RACE. For
24 the remaining PCRs a three-step protocol was used
25 where the annealing temperature of the first 5
26 cycles was 2°C higher than the lower T_m of the two
27 primers. In the second step the annealing
28 temperature of 5 cycles was the same as the lower
29 T_m of the two primers. The third step was 20
30 cycles with annealing temperatures 2°C below the
31 lower T_m of the two primers. For the 5' RACE a

1 poly-A sequence was added to 50 ng marmoset
2 pituitary cDNA produced with gene specific primers.
3 Products were purified (Qiagen) and subjected to
4 PCR. 2 µl of products of a first round PCR, using
5 primers S3 and A3, were used in a second round of
6 PCR using primers S4 and A4 (S3,
7 GACCACGCGTATCGATGTCGACTTTTTTTTTTTTTTTT (SEQ ID No
8 21) (anchored RACE primer from Boehringer
9 Mannheim); A3, GAAGGGACTGGACCAGCTCG (SEQ ID No 22);
10 S4, GACCACGCGTATCGATGTCGAC (SEQ ID No 23); A4,
11 CAAGGCAAGCAGGAACTAAG (SEQ ID No 24)). For the 3'
12 RACE 50 ng marmoset pituitary cDNA was produced
13 with anchored oligo-dT primers was subjected to PCR
14 using primers S5 and S3 (S5, ACCTCTTCACCTTCTGCTGCCT
15 (SEQ ID No 25)). 2 µl of products from this PCR
16 and primers S6 and S4 were used in a secondary PCR
17 (S6, CTCCTCAATGCTCCTTTGGATC (SEQ ID No 26)).
18 Products were size purified (Qiagen), cloned into
19 pGEM-T (Promega) and sequenced. Full length
20 marmoset Type II GnRH receptor was produced by PCR
21 using oligos of the 5' UTR (S7,
22 GAATTCGCTTCATACTCACACTTCATC (SEQ ID No 27); S8,
23 CGGAATTCTCACACTTCATCCTCCTATCTC (SEQ ID No 28)) and
24 the 3' sequence including the stop codon
25 (A5, GCTCTAGAGATCAGATTGATGTTATAGGAATG (SEQ ID No
26 29)). 50 ng of marmoset brain stem cDNA produced
27 with random hexa-polynucleotides was subjected to
28 PCR using primers S7 and A5. 2 µl of products and
29 primers S8 and A5 were used in a secondary round of
30 PCR. Products of this PCR were purified (Qiagen),
31 cloned into pcDNA3.1+ (Invitrogen) and sequenced.

1 The resultant plasmid was used in expression
2 studies.

3
4 **Immunocytochemistry.** Tissues from human, mouse,
5 sheep, rhesus and cynomologus monkeys were
6 obtained. An antiserum to the human Type II GnRH
7 receptor was produced by immunisation of rabbits
8 with a synthetic peptide corresponding to EC3
9 (YSPTMLTEVPPC (SEQ ID No 30)) conjugated to keyhole
10 limpet haemocyanin via the Cys residue. This
11 peptide, a synthetic peptide to EC3 of the human
12 Type I receptor (DPEMLNRLSDPC (SEQ ID No 31)) and
13 haemocyanin were used for immunoneutralisation
14 specificity studies. For detection of mammalian
15 GnRH I specific antiserum GF6 was used (18).

16
17 Tissue sections (15 μ m) were subjected to the
18 peroxidase/diaminobenzidine visualisation technique
19 as previously described (18, 19). Fluorescent
20 labelling was accomplished using the same procedure
21 up to the step prior to ABC reaction, when the
22 fluorescein label (Rhodamine 600, avidin D or FITC)
23 was applied to the slides and incubated at room
24 temperature in the dark for 2-4 hours. For double
25 labelling, slides were incubated sequentially with
26 avidin D and biotin blocking solutions for 15 min
27 each, then re-incubated with the next primary
28 antibody, followed by the other fluorescent
29 labelling (Rhodamine or FITC). Controls, including
30 omission of primary antibodies and order of
31 exposure, were consistently negative.

1 Immunofluorescence was viewed at two wave lengths
2 by confocal microscopy.

3
4 **Cell culture and transfection.** COS-7 cells were
5 cultured as previously described (20, 21).
6 Transient transfections of COS-7 cells with human
7 Type I GnRH receptor or marmoset Type II GnRH
8 receptor, along with myc-tagged ERK2, JNK and p38 α
9 constructs were performed using Superfect (Qiagen)
10 according to the manufacturer's protocol. All
11 assays described were performed 48 h post
12 transfection.

13
14 **Receptor binding and inositol phosphate production.**
15 Receptor binding utilising ^{125}I -[His⁵-D-Tyr⁶]GnRH I
16 and inositol phosphate production by GnRH ligands
17 were studied as previously described (20-22).

18
19 **Phospho-MAP kinase assay.** Serum starved (12-16 h)
20 COS-7 cells, transfected with human Type I or
21 marmoset Type II GnRH receptors were treated with
22 either mammalian GnRH I, GnRH II or antagonist 135-
23 18 for the time and dose specified for Fig. 9 at
24 37°C. After ligand stimulation, COS-7 cells were
25 prepared for immunoprecipitation (23). Co-
26 transfected myc-tagged MAP kinase constructs were
27 immunoprecipitated from cell lysates by overnight
28 incubation with myc-agarose slurry (Santa Cruz),
29 washed, an equal volume of 2x Laemmli sample buffer
30 added, resolved by SDS-PAGE and electrotransferred
31 to PVDF membrane (NEN Life Sciences). Activated MAP

1 kinase was detected using anti-phospho-
2 ERK/JNK/p38a-kinase specific antisera (New England
3 Biolabs), visualised by enzyme-linked
4 chemifluorescence (Amersham-Pharmacia) and
5 quantified using a phosphorimager. The degree of
6 phosphorylated MAP kinase was normalised to the
7 amount of unphosphorylated MAP kinase detected with
8 specific antisera.

9
10 **Expression of Type II receptor mRNA in marmoset and**
11 **human tissues.** Total RNA was extracted from
12 various marmoset tissues using TRI reagent (Sigma),
13 and cDNA was produced using oligo dT primers
14 (Ambion). PCR was performed on the cDNA using
15 marmoset Type II GnRH receptor cDNA specific
16 primers spanning exons 1-3 (sense:
17 CTCGGCTGGAGGGAACCTG, antisense:
18 GGTGCCCTCTTCGGCAGC), and actin specific primers.
19 PCR products were run on an agarose gel and blotted
20 onto HybondN⁺ nylon membrane (Amersham). The
21 Southern blot was probed with random primed
22 marmoset Type II GnRH receptor cDNA or actin cDNA.
23 Southern blots were quantified using a
24 phosphorimager and marmoset Type II GnRH receptor
25 expression was normalised to the expression of
26 actin.

27
28 Expression in human tissues was examined by
29 Northern blots of mRNA with random primed ³²P
30 labelled human Type II GnRH receptor exon 1. The
31 human Type II GnRH receptor genomic sequence (P1

1 clone) was obtained by Genome Systems (St Louis,
2 MD) using PCR screen of P1 clones with
3 oligonucleotides to human sequences (17).
4 Oligonucleotides (antisense: CTGTCCTGCCCCGGTCCTGAG;
5 sense: TGCCACCTTCTCGGCAGCA) to this sequence were
6 used with the P1 clone to produce a 460 bp
7 amplicon. Labelling was done with ^{32}P dCTP (6000
8 Ci/mMole) using supplier's specified conditions
9 (Stratagene). Hybridisation was performed using 2×10^7 cpm at 65°C in 5 x SSC/0.005% SDS/5x
10 Denhardt's/2 mg/ml salmon sperm DNA, washing with
11 0.1 x SSC/0.5% SDS at 55°C and the blots exposed to
12 X-ray film for 6 days.
13

14
15 **Stimulation of LH and FSH in sheep.** The relative
16 potency of mammalian GnRH I and GnRH II at inducing
17 FSH and LH secretion *in vivo* was tested using our
18 Soay ram sheep model (24). This was repeated
19 during both the sexually active (short days, SD)
20 and inactive phases (long days, LD) of the
21 photoperiod-induced reproductive cycle (24). The
22 same animals ($n = 8$) were used on the two
23 occasions. The GnRHs were administered at doses of
24 250 ng/ram and 10 µg/ram in a cross-over design
25 with a week between treatments to permit full
26 recovery. The peptides were dissolved in 1 ml of
27 0.9% saline and given as an intravenous bolus.
28 Blood samples were collected every 10 min, from 20
29 min before, until 2 h after the treatments and
30 assayed by specific radioimmunoassays (24). The
31 FSH and LH responses to the GnRH agonists were

1 calculated as delta responses (2 h mean hormone
2 concentration post treatment minus 20 min mean pre-
3 treatment hormone concentration). These values
4 were used to calculate the FSH : LH response ratio
5 for GnRH II and GnRH I, and thus the overall ratio
6 for the GnRH II stimulation compared with the
7 mammalian GnRH I stimulation. This was assessed
8 for each animal and then for the group (mean \pm SEM,
9 $n = 8$), under both LD and SD.

10

11 **Results and Discussion**

12

13 **Cloning and primary structure of the Marmoset Type** 14 **II GnRH receptor.**

15

16 Amplification of EC3 from genomic DNA from a range
17 of vertebrate species revealed two distinct
18 sequences of receptors representing the known Type
19 I receptors and novel Type II receptors in an
20 amphibian and reptile (25). Searches of human EST
21 databases revealed homologous sequences to the
22 reptile EC3 (17). From EST contigs we constructed
23 a partial receptor sequence encoding the putative
24 exons 2 and 3 corresponding to these exons of the
25 Type I receptor (17). All ESTs were in the
26 antisense orientation and it transpired that these
27 were in the 3' untranslated region (UTR) of a novel
28 human ribonucleoprotein (RBM8) which was highly
29 expressed in all tissues examined (26). The
30 equivalent of exon 1 was absent from the RBM8 3'
31 UTR. It was therefore evident that the

1 identification of sequences homologous to exon 1
2 was essential to discover the Type II receptors.
3 Searches of human databases, using as a query exon
4 1 of the human Type I receptor, revealed several
5 ESTs and genomic sequences BG 036291, AA 954764; AL
6 160282.

7
8 Since the receptor was likely to be a rare
9 transcript expressed in discrete tissues, we
10 generated antisera to the EC3 domain of the human
11 Type II receptor and found by immunocytochemistry
12 strong reactivity in pituitary and brain of the
13 human, rhesus monkey, sheep and mouse (Fig. 2). We
14 then used oligonucleotides to the human exon 1 and
15 RBM8 3' UTR sequences to amplify cDNA from marmoset
16 pituitary and brain by PCR and 5' RACE procedures.
17 The full length cDNA encodes a 380 amino acid
18 protein with characteristic G protein-coupled
19 receptor (GPCR) structure SEQ ID No. 1. Although
20 it is more homologous with GnRH receptors than
21 other GPCRs, it has only 41% sequence identity with
22 the human Type I receptor suggesting an early
23 evolutionary gene duplication. It also possesses a
24 carboxy terminal tail which is important for rapid
25 desensitization and is uniquely absent from
26 mammalian Type I receptors (27-30). The receptor
27 also does not have the unusual Asn/Asp microdomain
28 of transmembrane helices 2 and 7 of the mammalian
29 Type I receptors which plays a role in receptor
30 activation (21). Instead it has the Asp/Asp motif
31 as in non-mammalian Type I GnRH receptors recently

1 cloned (7, 9). The *Drosophila* GnRH receptor
2 homologue has the usual Asp/Asn motif
3 characteristic of most GPCRs (31) indicating that
4 there was an initial mutation to Asp/Asp in the
5 ancient vertebrate GnRH receptor followed by
6 mutation to Asn/Asp in the mammalian Type I
7 receptors. The activation role of this microdomain
8 (21) may therefore be further elucidated by
9 experimentation with the Type II receptor. The
10 LSD/EP sequence of EC3 which is important for
11 ligand selectivity of mammalian Type I receptors
12 (9, 20) is replaced by VPPS which is also present
13 in reptile (VPPS) and amphibian (VPPV) Type II GnRH
14 receptors (25). This difference in sequence is
15 likely, therefore, to be a determinant of Type II
16 receptor selectivity for GnRH II as all other
17 binding sites (9) are conserved.

18

19 **Pharmacological characterization of the marmoset**
20 **Type II GnRH receptor.**

21 Fig. 4 shows the heterologous competition binding
22 of ^{125}I -[His⁵, D-Tyr⁶]GnRH mediated by the Marmoset
23 Type II GnRH receptor expressed in the transfected
24 COS-7 cells and shows that the Type II GnRH
25 receptor demonstrates high selectivity for GnRH
26 Type II.

27

28 The affinity and specificity of the Type II GnRH
29 receptor for the natural GnRHs and two GnRH
30 analogues is shown in Fig. 5. Further
31 characterisation of GnRH agonists and antagonists

1 can be determined with the routine binding assays.
2 The identification of the signalling pathway of the
3 receptor was examined by testing the ability of the
4 ligand-induced receptor to activate second
5 messenger generating systems.

6
7 Using the above described expression system, we
8 have determined that the Type II GnRH-R activates
9 the production of Inositol phosphate (see Fig. 5)
10 but does not stimulate cyclic AMP production.

11
12 Thus, expression of the Type II receptor in COS-7
13 cells revealed that it is highly selective for GnRH
14 II in receptor binding assays (Fig. 4) and in the
15 stimulation of inositol phosphate intracellular
16 messenger production (40-fold and 90-fold greater
17 activity relative to mammalian GnRH I) (Fig. 5)
18 (Table 1). This contrasts with the Type I receptor
19 in which GnRH II has only 10% and 9% activities of
20 mammalian GnRH I in these assays (Fig. 6). Overall
21 GnRH II has an affinity 24-fold greater for the
22 Type II receptor than for the Type I receptor. The
23 Type II receptor was also more selective for salmon
24 GnRH and [D-Arg⁶]GnRH II (Table 1).

25

26

27

28

29

Table 1 : Comparative ligand binding and inositol phosphate production properties of marmoset type II & human Type 1 GnRH receptors.

Peptides	Ligand binding (IC ₅₀)		InsP production (EC ₅₀)	
	Marmoset Type II	Human Type I	Marmoset Type II	Human Type I
GnRH II	1.07±0.04	26.1±4	0.45±0.05	7.41±1.55
GnRH I	42.6±3.19	2.81±0.17	40.5±4.43	0.63±0.08
sGnRH	9.48±2.17	244±23.6	5.99±0.91	9.62±3.5
[D-Arg ⁶] GnRH II	3.34±0.06	11.9±0.35	2.39±0.64	3.8±0.71
Antagonist 135-18	1650±478	10.6±1.4	276±45.5	Full antagonist

Data are expressed in nanomolar and represent the s.e.m. (n=3-6). Mammalian GnRH I (GnRH I) salmon GnRH (sGnRH: [Trp⁷,Leu⁸]GnRH I), GnRH II ([His⁵,Trp⁷,Tyr⁸]GnRH I)

1 Moreover, a Type I receptor GnRH antagonist behaved
 2 as an agonist at the Type II receptor (Fig. 5). It
 3 has recently been demonstrated that control of
 4 gonadotropin biosynthesis and regulation of
 5 gonadotrope function regulated by GnRH can be
 6 mediated by the activation of mitogen-activated

1 protein kinases (MAP kinases). Therefore we
2 assessed the capacity of both the Type I human GnRH
3 receptor and the marmoset Type II GnRH receptor to
4 activate the three major MAP kinase prototypes,
5 ERK, JNK (a particular type of MAP kinases
6 available through Eisuke Nishida, Kyoto University)
7 and p38 α in COS-7 cells. At the Type I receptor,
8 mammalian GnRH I was considerably more potent than
9 GnRH II in activating ERK2 (Fig. 8a). In contrast,
10 at the Type II receptor the ligand specificity was
11 the inverse and antagonist 135-18 had significant
12 agonist activity compared with low activity at the
13 Type I receptor (Fig. 8 panels a and b). Agonist-
14 induced activation of JNK was not detected with
15 stimulation of either the Type I or Type II
16 receptor (data not shown). However, activation of
17 p38 α was detected upon stimulation of the Type II
18 receptor but not with stimulation of the Type I
19 receptor (Fig. 8c). The time course of p38 α
20 activation was also considerably more protracted
21 than that for Type I/II receptor activation of
22 ERK2. In addition we noted that ERK2 stimulation
23 via the Type I GnRH receptor is more transient than
24 that mediated by Type II GnRH receptor stimulation
25 (Fig. 8c). There are therefore distinct
26 differences in signalling by the two receptors.
27
28 **Tissue distribution and expression of the marmoset**
29 **Type II GnRH receptor.** To gain insight into the
30 potential functions of the Type II receptor, its
31 expression in human and marmoset tissues was

1 examined. PCR amplification of cDNA from marmoset
2 brain tissues revealed that it is expressed in the
3 pituitary, spinal cord, pons, cerebellum, putamen,
4 medulla, hypothalamus, preoptic area, midbrain,
5 occipital pole, frontal lobe and corpus callosum
6 (Fig. 9a). Expression was high in reproductive
7 tissues such as testis, prostate, mammary glands,
8 seminal vesicles and epididymis. Substantial
9 expression was detected in adrenal, thyroid, heart
10 and skeletal muscle but little or no expression was
11 found in other tissues such as liver, ovary and
12 bladder (Fig. 9a). Northern blots yielded a
13 similar expression pattern (data not shown).
14 Northern blots on human tissues probed with exon 1
15 showed highest expression in the cerebral cortex
16 and occipital pole, moderate expression in the
17 frontal lobe, temporal lobe and putamen, and low
18 expression in the cerebellum, medulla and spinal
19 cord (Fig. 9b). There was substantial expression
20 in the amygdala and low expression in the caudate
21 nucleus, corpus callosum, hippocampus, substantia
22 nigra, subthalamic nucleus and thalamus (data not
23 shown). There was also significant expression in
24 the heart and pancreas but little or no expression
25 in placenta, lung, liver, skeletal muscle and
26 kidney (Fig. 9c).

27

28 **Type II GnRH receptor function in the pituitary.**

29 A specific antiserum to EC3 of the human Type II
30 receptor was used to conduct immunocytochemistry
31 and demonstrated specific expression of the

1 receptor in human anterior pituitary (Fig. 2). In
2 view of the possibility that the novel Type II
3 receptor may regulate pituitary function, we
4 determined if it was expressed in the pituitary of
5 other mammals. Staining was also found in about
6 10% of cells, (the relative occurrence of
7 gonadotropes), in the anterior pituitary of the
8 mouse and sheep (Fig. 2). In the sheep anterior
9 pituitary double staining with Type II receptor and
10 LH antisera revealed that the Type II receptor
11 immunoreactivity is co-localised in 69% of LH
12 positive cells (Fig. 2). Only 12% of Type II
13 receptor positive cells were negative for LH.
14 Since mammalian GnRH I binding sites also co-
15 localise with LH in up to 90% of gonadotropes in
16 the rat pituitary at proestrus (32), it is likely
17 that the majority of gonadotropes express both Type
18 II and Type I receptors and suggests that these
19 receptors may co-ordinately regulate LH and FSH
20 biosynthesis and secretion. The presence of Type
21 II receptors in the majority of gonadotropes is, at
22 first consideration, unexpected as there is a
23 substantial literature suggesting that a single
24 GnRH (mammalian GnRH I) is sufficient to regulate
25 the secretion of gonadotropins, and that the
26 differential secretion of LH and FSH during the
27 mammalian ovarian cycle may be adequately accounted
28 for by modulatory effects of gonadal steroids
29 (androgen, estrogen and progesterone) and peptides
30 (activin, inhibin and follistatin) (14). However,
31 a substantial number of physiological studies

1 invoke the existence of an FSH-releasing peptide to
2 account for the differential secretion of
3 gonadotropins (13-16).

4

5 In the early studies on the GnRH II (previously
6 called chicken GnRH II), it was found to have
7 preferential FSH-releasing activity when compared
8 with chicken GnRH I (chicken Type I GnRH) (33).

9 Moreover, GnRH II has been localised to the
10 hypothalamic area in species of non-mammalian
11 vertebrates (see Refs. (7, 8) for review) and the
12 supraoptic, paraventricular, arcuate and pituitary
13 stalk regions of monkeys where it is thought to
14 play a role in gonadotropin secretion (19, 34). We
15 therefore conducted studies using a well-
16 established sheep model to determine the relative
17 effects of mammalian GnRH I and GnRH II on LH and
18 FSH secretion. The responses to a 250 ng bolus of
19 GnRHs was too low for comparison of relative LH and
20 FSH secretion. At the 10 µg dose all the rams
21 showed a robust response and every individual
22 exhibited a higher ratio of FSH to LH secretion
23 when treated with GnRH II compared with mammalian
24 GnRH I (Fig. 7). The mean ratio of FSH to LH
25 induced by GnRH II was 2.14 ± 0.29 and 2.02 ± 0.34
26 (mean \pm SD) times higher than that induced by
27 mammalian GnRH I for sexually active and sexually
28 quiescent rams respectively. The FSH/LH ratios
29 generated in both sexually active and quiescent
30 rams upon GnRH II treatment were both significantly
31 greater ($p=0.03$, $p=0.002$ respectively, paired two-

1 tailed t-test) than with mammalian GnRH I
2 treatment.

3

4 When it is considered that GnRH II has an affinity
5 and potency of $\leq 20\%$ of mammalian GnRH I at the
6 Type I receptor (7, 9, 20), and the *in vivo*
7 secretion of the two peptides is likely to be
8 finely tuned in both concentration and phasing of
9 pulsatile release, our exogenous bolus
10 administration of the peptides is a relatively
11 crude approach. Thus, the relative differential
12 stimulation of FSH by endogenously secreted GnRH II
13 may be much greater *in vivo*.

14

15 The findings of GnRH II in the hypothalamus, the
16 presence of the Type II receptor immunoreactivity
17 in gonadotropes and differential release of
18 gonadotropins suggest that, contrary to existing
19 dogma, gonadotropins are regulated by two different
20 forms of GnRH acting through two separate cognate
21 gonadotrope receptors. In order to elicit
22 differences in relative LH and FSH secretion
23 mammalian GnRH I and GnRH II would have to have
24 different patterns of duration of release,
25 concentration and pulse frequency of secretion
26 and/or differential intracellular signalling
27 pathways. The differential, temporal or
28 qualitative, downstream signalling between the Type
29 II and Type I receptors shown here may provide the
30 means for preferential FSH secretion.

31

1 GnRH II activation of the Type II receptor in
2 bullfrog sympathetic ganglia potently inhibits M-
3 type K⁺ channels (11). A similar action in
4 gonadotropes would partially depolarise them thus
5 facilitating external excitatory inputs to the cell
6 or entry of extracellular Ca²⁺ through L type
7 channels, which occurs on stimulation of Type I
8 receptors by mammalian GnRH I (1-3). These two
9 GnRHs and GnRH receptor systems, along with
10 differences in signalling pathways, provide the
11 means for differential FSH and LH secretion and
12 open the possibility of developing new GnRH II
13 analogues for the treatment of diseases of the
14 reproductive system as well as contraceptives which
15 selectively inhibit FSH and gametogenesis without
16 affecting sex steroid hormone production.

17

18 **Type II GnRH receptor may have roles in neural**
19 **development and sexual arousal.** In view of its
20 wide distribution in the central and peripheral
21 nervous systems, GnRH II has been proposed to have
22 a neuromodulatory role (7, 8) as evidenced by K⁺
23 channel inhibition in bullfrog sympathetic ganglia
24 (11). Our demonstration of a GnRH II-selective
25 receptor expression in many brain regions (Figs. 2,
26 9) supports this. Type II GnRH receptor antisera
27 immunoreactive cells were widely seen in the
28 extrahypothalamic regions, such as medial septum,
29 bed nucleus of the stria terminalis, medial
30 preoptic area, substantia innominata, basal nuclues
31 of Meynert, claustrum, amygdala and putamen, and in

1 the hypothalamic regions, such as supraoptic
2 nucleus, periventricular area, ventromedial nucleus
3 and dorsomedial nucleus in rhesus monkeys at
4 embryonic days E58, E70 and E78 as well as in the
5 adult cynomologus monkey. In some of these areas
6 (e.g. midbrain and supraoptic nucleus) the GnRH II
7 ligand is also expressed (19, 34). The
8 distribution pattern of Type II GnRH receptor
9 positive cells in extrahypothalamic regions
10 overlapped with that of the early developing
11 mammalian GnRH I cells we have described (18).
12 Later in embryonic development the GnRH I cells
13 were not consistently immunopositive with Type II
14 GnRH receptor antiserum. This suggests a potential
15 role for the receptor in the development of
16 mammalian GnRH I neurones. An intriguing
17 observation was that neurones which express the
18 mammalian GnRH I gene in the preoptic area and
19 periventricular region of the hypothalamus (Ref.
20 34) were stained with the Type II GnRH receptor
21 antiserum in the rhesus monkey, suggesting that
22 GnRH II may regulate mammalian GnRH I neurones.
23 Mammalian GnRH I is known to have ultrashort
24 feedback on its own secretion (8, 35) but the co-
25 localisation of Type II receptor on mammalian GnRH
26 I neurones in the hypothalamic regions suggests
27 that some effects on mammalian GnRH I secretion may
28 be mediated via GnRH II.
29 GnRH has been shown to have direct effects on
30 reproductive behaviour and sexual arousal in
31 rodents independent of its stimulation of sex

1 hormone production (8, 36). Rapid changes in GnRH
2 content of brain areas, cell number and cell size
3 in response to visual, olfactory and other
4 stimulants of sexual behaviour have been observed
5 in species of fish, amphibians, reptiles and
6 mammals (see Refs. (7, 8, 10, 36, 37) for review).
7 Moreover, GnRH II is much more effective than
8 mammalian GnRH I in stimulating courtship and song
9 in ring doves (7) and song sparrows (12), and GnRH
10 II distribution shifts from midbrain cell bodies to
11 terminal regions following the initiation of
12 courtship in newts (37). There is remarkable
13 concurrence of the distribution of the Type II GnRH
14 receptor in the temporal lobe, putamen, amygdala,
15 medial preoptic area, ventromedial nucleus,
16 dorsomedial nucleus and periventricular nucleus of
17 the human or monkey brain with effects of lesions
18 and/or electrical stimulation of these areas on
19 reproductive behaviours such as sexual interest,
20 erection, intromission, thrusting and ejaculation
21 in rats, dogs, cats, monkeys and humans (36-38).
22 GnRH II has also been localised to these regions in
23 the rhesus monkey (19, 34).

24

25 The mechanism of action of GnRH II in the nervous
26 system of mammals is unknown but the peptide has
27 been identified in sympathetic ganglia of
28 amphibians where it binds to selective high
29 affinity receptors (22) and potently inhibits M-
30 type K^+ channels (11). Inhibition of these K^+
31 channels by GnRH II facilitates fast excitatory

1 transmission by conventional neurotransmitters, by
2 increasing input resistance of postsynaptic
3 neurones and by partial depolarization (11). This
4 may, therefore, provide a general neuromodulatory
5 mechanism for GnRH II effects in the nervous
6 system, and specifically in reproductive behaviour,
7 by facilitating signalling by neurotransmitters.

8
9 **Type II GnRH receptor in reproductive tissues.** The
10 marmoset Type II GnRH receptor expression and GnRH
11 II ligand expression (7, 8, 11) in non-neural
12 reproduction-related tissues such as the mammary
13 gland, prostate, gonads and adrenal cells may
14 resolve the long-standing enigma of the non-
15 concurrence of the binding pharmacology of
16 receptors in these tissues and in various tumours
17 (e.g. prostate, ovarian and mammary gland (1-3))
18 with that of the known pituitary Type I receptor
19 which is believed to be the receptor in these
20 tissues. For example, the paradox of similar
21 effects of both GnRH agonists and antagonists (1,
22 2) on proliferation of these tumour cell lines can
23 be rationalised if the Type II receptor is
24 mediating these effects, as we have shown that
25 certain mammalian GnRH I antagonists (e.g. 135-18)
26 behave as agonists with the Type II receptor (Fig.
27 8b). Moreover, the antiproliferative effects of
28 GnRH analogues on cell lines of these tumours is
29 consistent with the activation of p38 α by the Type
30 II receptor since this MAP kinase is known to be
31 antiproliferative (39).

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1 CLAIMS

2

3 1. A polynucleotide encoding a functional Type II
4 gonadotropin-releasing hormone receptor (Type II
5 GnRH-R) peptide.

6

7 2. A polynucleotide as claimed in Claim 1 encoding
8 at least a portion of exon I of Type II GnRH-R.

9

10 3. A polynucleotide as claimed in either one of
11 Claims 1 and 2 which encodes mammalian Type II
12 GnRH-R.

13

14 4. A polynucleotide as claimed in Claim 3 which
15 encodes a primate Type II GnRH-R.

16

17 5. A polynucleotide as claimed in Claim 4 which
18 encodes marmoset Type II GnRH-R.

19

20 6. A polynucleotide as claimed in Claim 5 having
21 the nucleotide sequence as set out in SEQ ID No.
22 1 or which has over 90% homology thereto.

23

24 7. A polynucleotide as claimed in Claim 4 which
25 encodes human Type II GnRH-R.

26

27 8. A polynucleotide as claimed in Claim 7 which
28 comprises a nucleotide sequence as set out in
29 any one of SEQ ID Nos. 3, 5, 7 or 9 or which has
30 over 90% homology thereto.

31

50

- 1 9. A polynucleotide is claimed in Claim 8 which
2 encodes an amino acid sequence as set out in any
3 one of SEQ ID Nos. 4, 6, 8 or 10 or which has
4 over 90% homology thereto.
5
- 6 10. A polynucleotide as claimed in any one of Claims
7 1 to 9 which encodes a peptide able to bind
8 specifically to Type II GnRH.
9
- 10 11. A polynucleotide as claimed in Claim 10 which
11 encodes a peptide able to act as a receptor for
12 Type II GnRH-R.
13
- 14 12. A recombinant genetic construct comprising a
15 polynucleotide as claimed in any one of Claims 1
16 to 11.
17
- 18 13. An expression vector comprising a polynucleotide
19 as claimed in any one of Claims 1 to 11 and able
20 to express functional Type II GnRH-R peptide.
21
- 22 14. A host cell transformed with a vector as claimed
23 in Claim 13.
24
- 25 15. A host cell as claimed in Claim 14 able to
26 express functional Type II GnRH-R.
27
- 28 16. A transgenic animal having a construct as
29 claimed in Claim 12 stably integrated into its
30 genome.
31

51

- 1 17. A peptide comprising at least a portion of exon
2 I of Type II gonadotropin-releasing hormone
3 receptor (Type II GnRH-R).
4
- 5 18. An isolated functional Type II gonadotropin-
6 releasing hormone receptor (Type II GnRH-R).
7
- 8 19. A peptide as claimed in either one of Claims 17
9 and 18 which comprises a portion of exon I of
10 mammalian Type II GnRH-R.
11
- 12 20. A peptide as claimed in Claim 19 which comprises
13 a portion of exon I of primate Type II GnRH-R.
14
- 15 21. A peptide as claimed in any one of Claims 17 to
16 20 having an amino acid sequence as set out in
17 SEQ ID No. 2 which has over 90% homology
18 thereto.
19
- 20 22. A peptide as claimed in any one of Claims 17 to
21 20 having an amino acid sequence as set out in
22 any one of SEQ ID Nos. 4, 6, 8 or 10, or which
23 has over 90% homology thereto.
24
- 25 23. A peptide as claimed in any one of Claims 17 to
26 22 which is able to bind Type II GnRH
27 specifically.
28
- 29 24. A peptide as claimed in Claim 23 which is a
30 functional receptor for Type II GnRH.
31

- 1 25. An antibody able to bind specifically to Type II
2 GnRH-R.
3
- 4 26. An antibody as claimed in Claim 25 which is
5 specific to an extracellular domain EC1, EC2 or
6 EC3 of Type II GnRH-R.
7
- 8 27. A method of screening an agent for
9 pharmacological activity, said method
10 comprising:
11 a) providing functional Type II GnRH-R peptide
12 and exposing said peptide to the agent; and
13 b) ascertaining whether said agent interacts
14 with said Type II GnRH-R peptide.
15
- 16 28. The method as claimed in Claim 27 wherein said
17 Type II GnRH-R is expressed by a host cell as
18 claimed in Claim 15.
19
- 20 29. The method as claimed in Claim 27 wherein said
21 Type II GnRH-R is expressed by a host cell
22 transformed with a polynucleotide as claimed in
23 Claim 11 wherein said host cell imitates Type
24 II GnRH-R signal transduction at least
25 partially and wherein exposure of said host
26 cell to said agent results in Type II GnRH-R
27 signal transduction when said agent binds
28 successfully to said Type II GnRH-R peptide.
29
- 30 30. A method of inhibiting binding of GnRH to its
31 native receptor *in vivo*, said method comprising

1 administering Type II GnRH-R or an
2 extracellular domain thereof.

3

4 31. The method of Claim 30 wherein the EC2 loop of
5 Type II GnRH-R is administered.

6

7 32. A method of contraception, said method
8 comprising administering exogenous Type II
9 GnRH-R or an extracellular domain thereof to a
10 patient in quantities sufficient to
11 substantially diminish binding of endogenous
12 Type II GnRH to endogenous Type II GnRH-R.

13

14 33. Use of Type II GnRH-R or an extracellular
15 domain thereof to inhibit endogenous Type II
16 GnRH binding to its native receptor *in vivo*.

17

18 34. Use of Type II GnRH-R as a contraceptive.

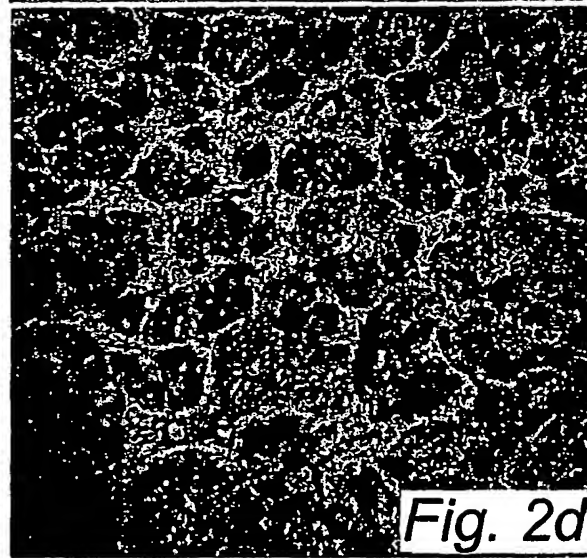
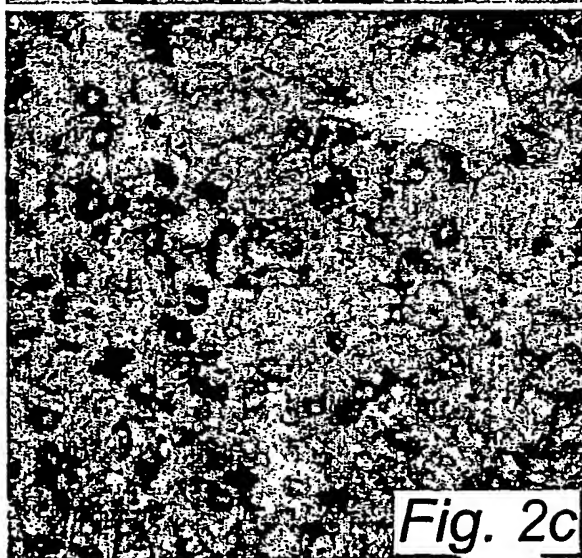
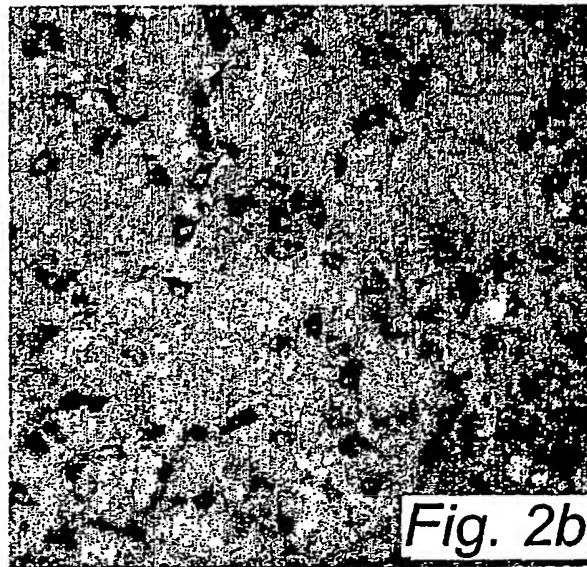
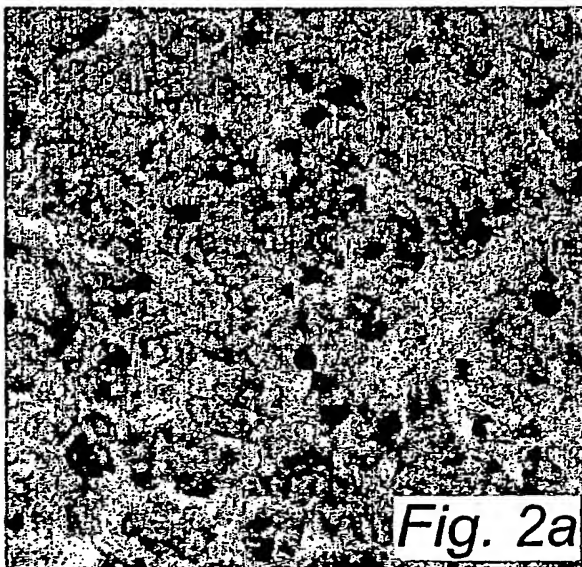
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20 35. The use as claimed in either one of Claims 33
21 and 34 wherein said Type II GnRH-R is expressed
22 from a recombinant genetic construct.



Fig. 1

2 / 11



3 / 11

```
HII  
MSAGNGTPW--AAGEEVWAGSGVEEGSELPTFSAAKVRVGVTIVLFVSSAGGNLAVLWSVTRRREPSQLRPCPVRR  
*** ** *|* *** ** *|*****|*****|*****|*****|*****|*****|*****  
  
MII  
MSAVNGTWPWGSSAREEVWAGSGVEEGSELPTFSTA KVRVGVTIVLFVSSAGGNLAVLWSVTRPQPSQLRPSPVRR
```

Frame shift in human sequence corrected by a short splice.

	80	90	100	110	120	130	140	150
HII								
LEFIHLAAADLLVTFVVMPLDATWNI								
TVQWLAVDIACRTLMELKIMATYSA								
FLPVVIGLDRQA								
AVLNPLGSRSGVRK								
** *****								
***** * *****								

MI I								
LEFAHLAAADLLVTFVVMPLDATWNI								
TVQWLAGDIACRTLMELKIMAMYAA								
FLPVVIGLDRQA								
AVLNPLGSRSGVRK								

Fig. 3

4 / 11

[illegible]

Δ

exon 2-3											
splice											
230	240	250	260	270	280	290	300				
HII											
RPQTRKGSHPAGEFALPRSFDCPRVRLRALRLALLVLLTFILCWTPYYLLGMWYWFSPMTLTVPPSLSHILFL											
* ***** ***** ***** ***** ** ***** *****											
MII											
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IV

Fig. 3 (cont'd)

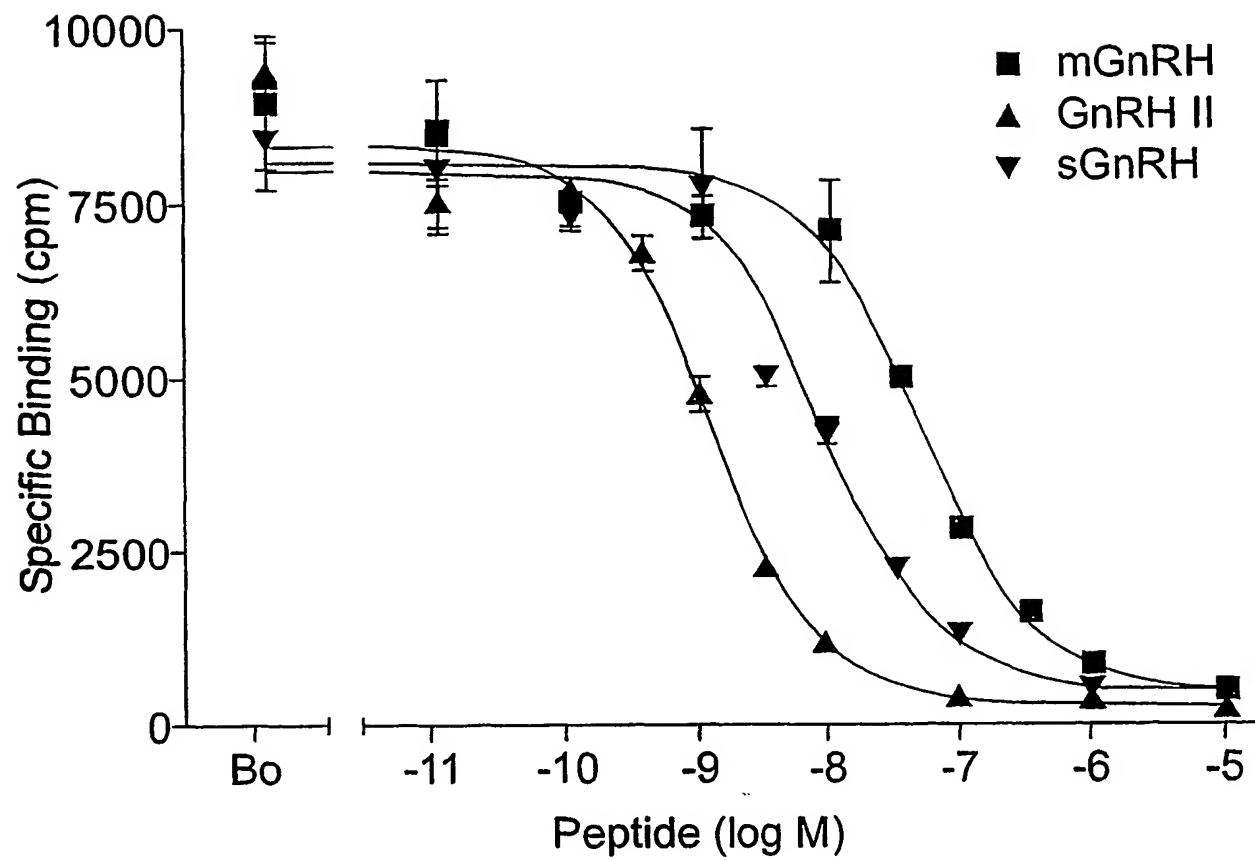
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	310		320		330		340		350		360		370
HII	GLLNAPLDPLLYGAFTLGCRRGHQELSIDSKE-GSGRMLQEEIHAFRQLEVQKTVTTSRRAGETKGISITSI												
	*****		*****		*****		*****		*****		*****		*****
	*****		*****		*****		*****		*****		*****		*****
MII	GLLNAPLDPLLYGAFTLGCRRGHQELSMDSSREEGSRRMFQDDIQALRQTEVQKTVTTSRKAGETKDIPITSI												

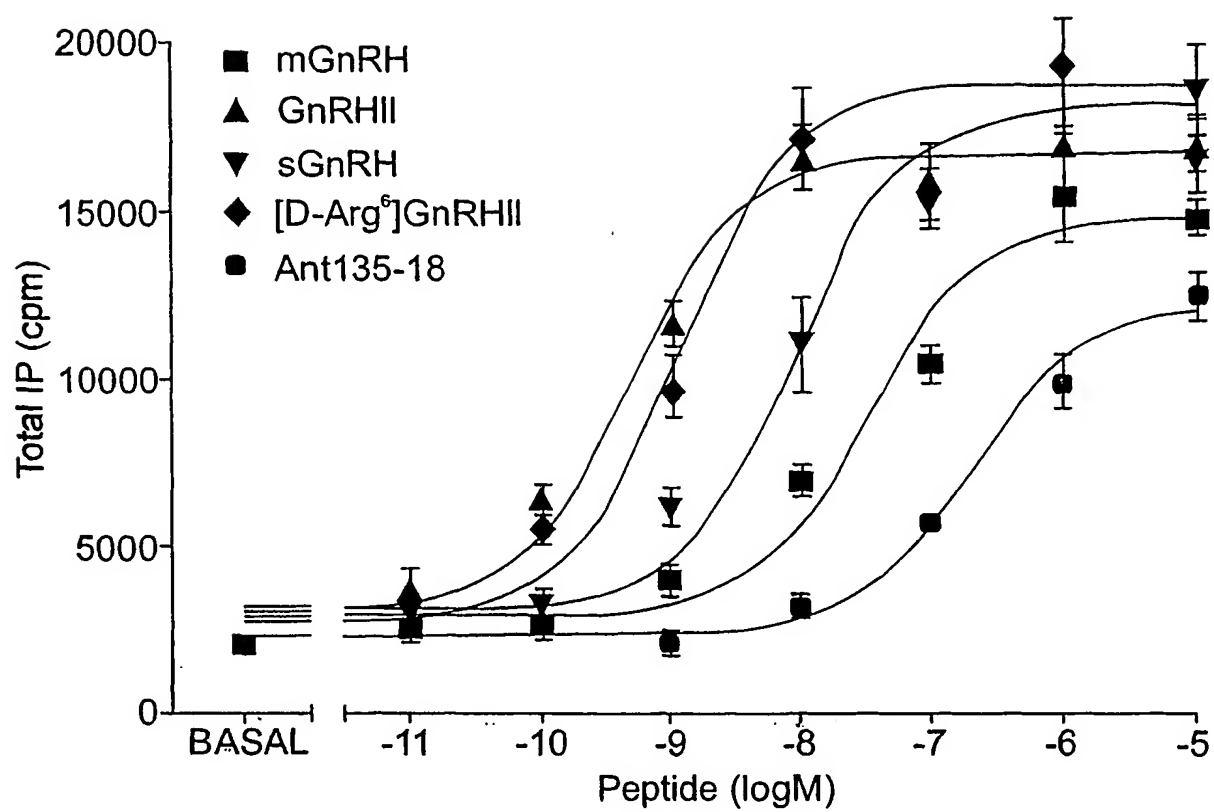
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Fig. 3 (cont'd)

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*Fig. 4*

7 / 11

*Fig. 5*

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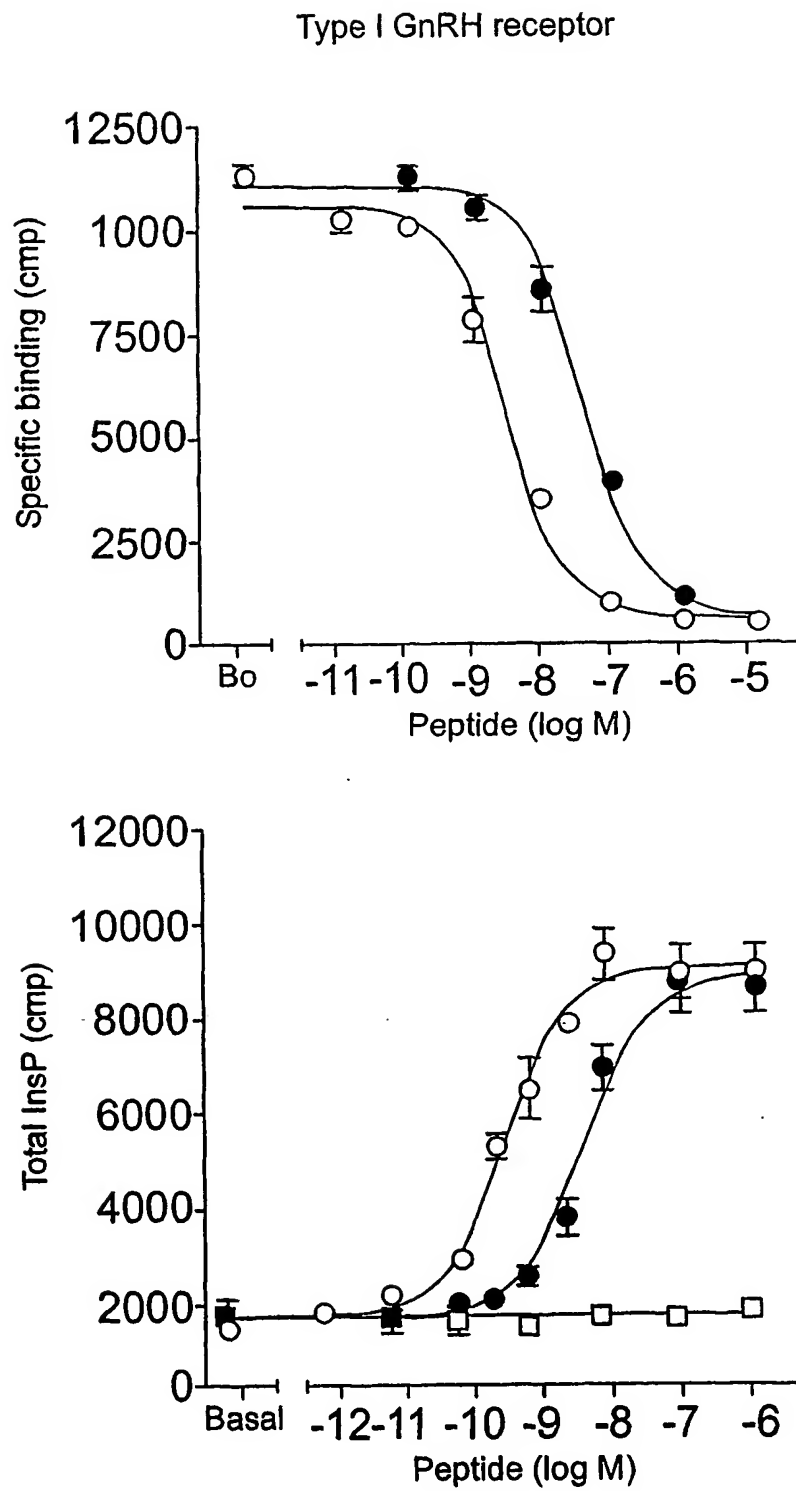
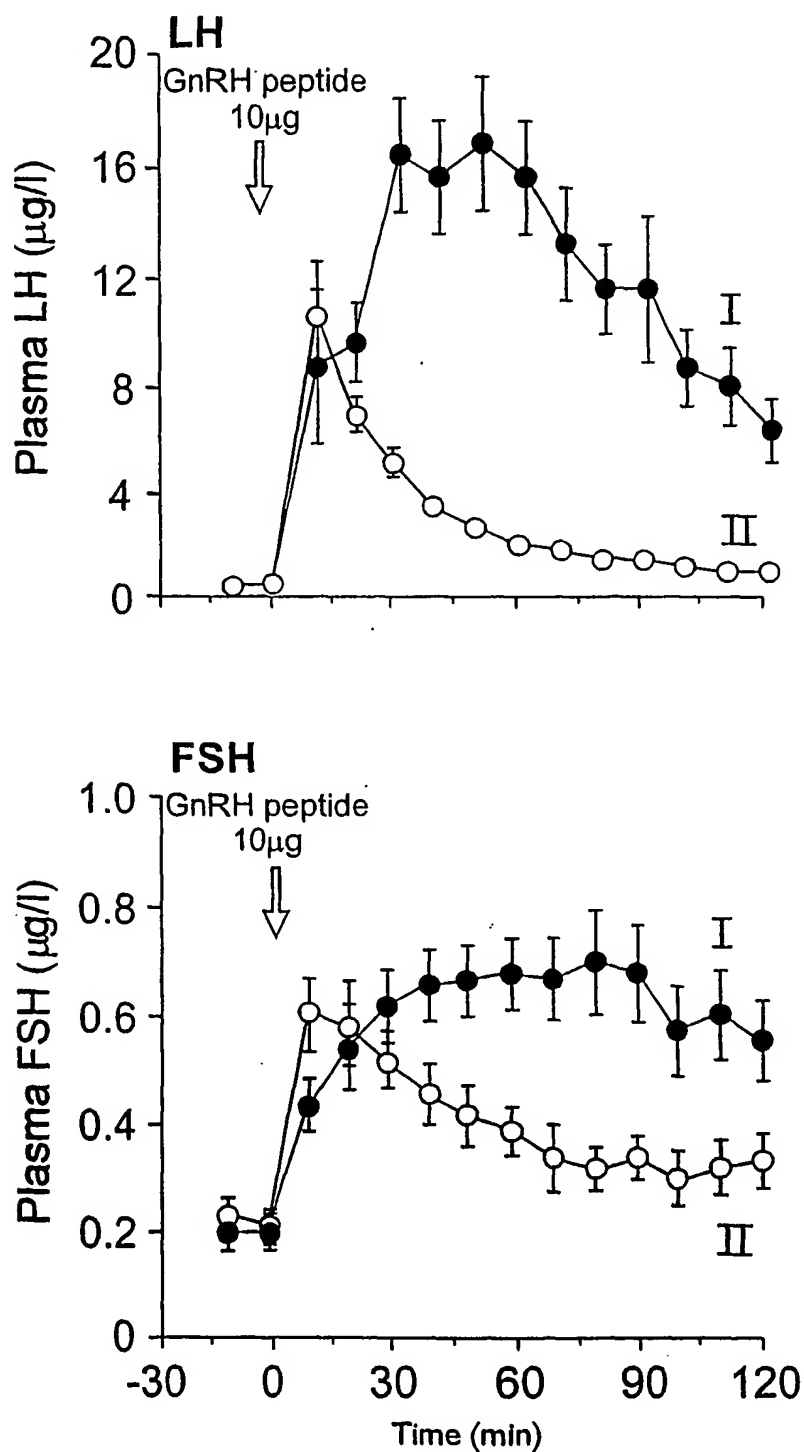


Fig. 6

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LH and FSH response to GnRH I & II -sheep

**Fig. 7**

10 / 11

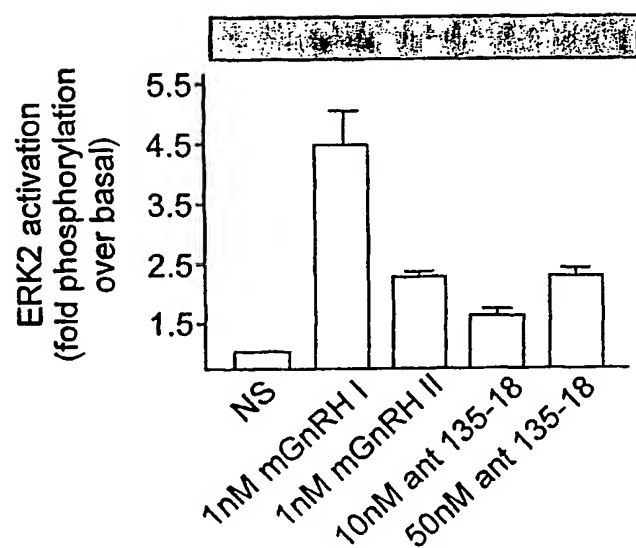


Fig. 8a

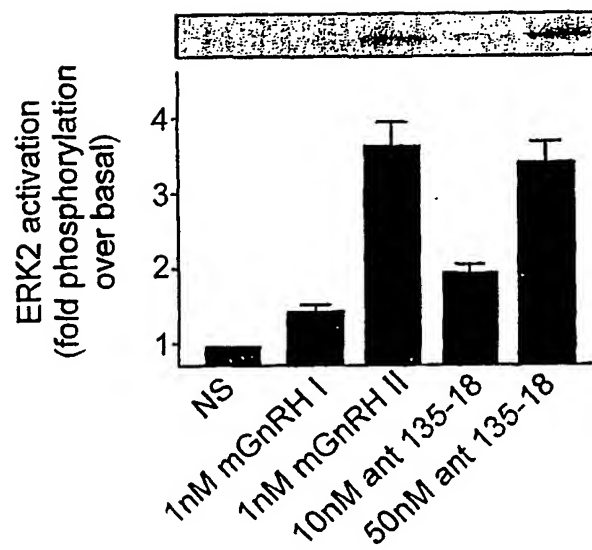


Fig. 8b

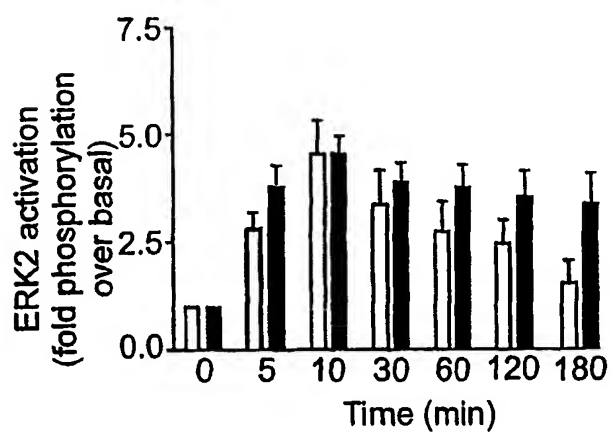
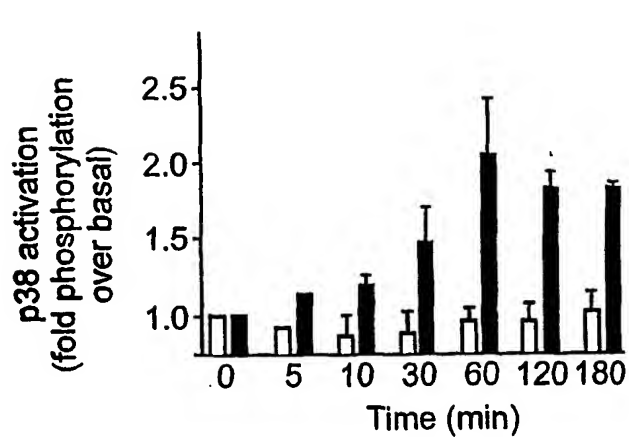
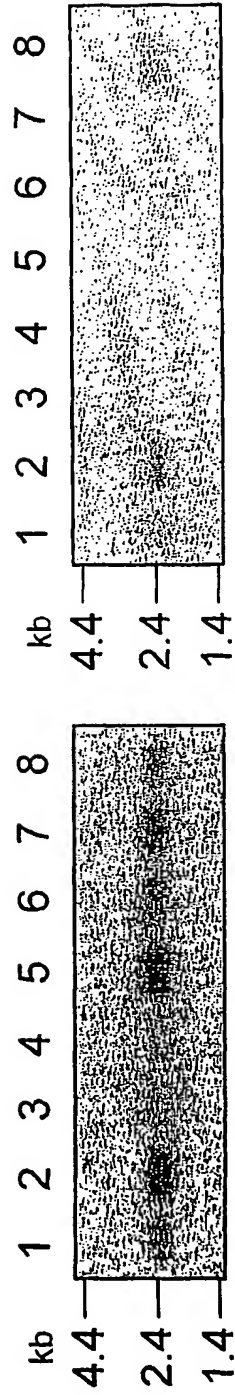
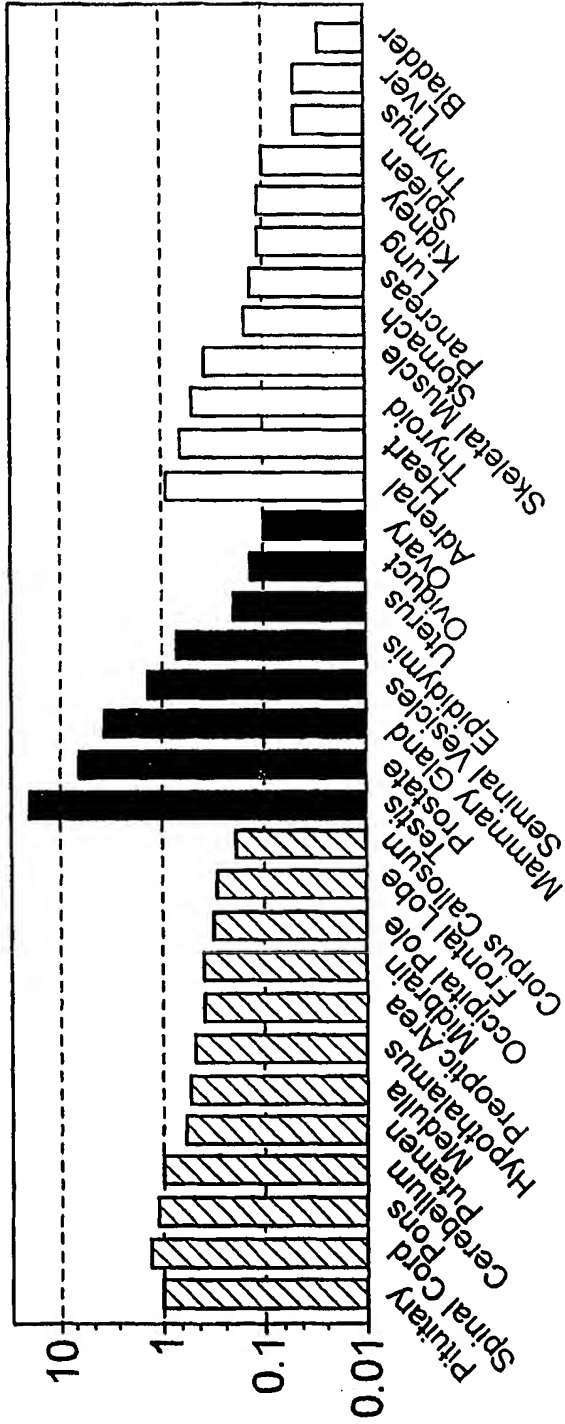


Fig. 8c

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SEQUENCE LISTING

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<130> P25556B

<160> 35

<170> PatentIn version 3.0

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Met Ser Ala Val Asn
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ggc acc cct tgg ggg tcc tca gcg cgg gag gag gtc tgg gca gga tcg      103
Gly Thr Pro Trp Gly Ser Ser Ala Arg Glu Glu Val Trp Ala Gly Ser
10 15 20

```

```

gga gtg gag gtg gag ggc tca gag ctg ccc acc ttc tcg aca gca gca      151
Gly Val Glu Val Glu Gly Ser Glu Leu Pro Thr Phe Ser Thr Ala Ala
25 30 35

```

```

aag gtc cga gtg gga gtg acc att gtg ctg ttt gtt tct tcg gct gga      199
Lys Val Arg Val Gly Val Thr Ile Val Leu Phe Val Ser Ser Ala Gly
40 45 50

```

```

ggg aac ctg gct gtc ctg tgg tca gtg aca cgg ccg caa ccc agc cag      247
Gly Asn Leu Ala Val Leu Trp Ser Val Thr Arg Pro Gln Pro Ser Gln
55 60 65

```

```

ctc cgc ccc tct ccg gtc agg aga ctc ttc gcc cat tta gca gcc gcc      295
Leu Arg Pro Ser Pro Val Arg Arg Leu Phe Ala His Leu Ala Ala Ala
70 75 80 85

```

```

gac tta cta gtc act ttt gtg gtt atg ccc cta gat gcc acc tgg aat      343
Asp Leu Leu Val Thr Phe Val Val Met Pro Leu Asp Ala Thr Trp Asn
90 95 100

```

```

atc act gtt cag tgg ctg gct ggg gac atc gca tgt cgg aca ctc atg      391
Ile Thr Val Gln Trp Leu Ala Gly Asp Ile Ala Cys Arg Thr Leu Met
105 110 115

```

```

ttc ctg aaa cta atg gcc atg tat gct gca gct ttc ctg cct gtg gtc      439
Phe Leu Lys Leu Met Ala Met Tyr Ala Ala Ala Phe Leu Pro Val Val
120 125 130

```

```

att gga ctg gat cgc cag gca gca gta ctc aat ccg ctt gga tcc cgc      487
Ile Gly Leu Asp Arg Gln Ala Ala Val Leu Asn Pro Leu Gly Ser Arg
135 140 145

```

```

tca ggt gta agg aaa ctt ctg ggg gca gcc tgg gga ctt agt ttc ctg      535
Ser Gly Val Arg Lys Leu Leu Gly Ala Ala Trp Gly Leu Ser Phe Leu
150 155 160 165

```

```

ctt gcc ttg ccc cag ctg ttc ctg ttc cat acc gtc cac cga gct ggt      583
Leu Ala Leu Pro Gln Leu Phe Leu Phe His Thr Val His Arg Ala Gly
170 175 180

```

cca gtt ccc ttc act cag tgt gcc acc aaa ggg agc ttc aag gct cga	631
Pro Val Pro Phe Thr Gln Cys Ala Thr Lys Gly Ser Phe Lys Ala Arg	
185 190 195	
tgg caa gag acc acc tat aac ctc ttc act ttc tgc tgc ctc ttt ctg	679
Trp Gln Glu Thr Thr Tyr Asn Leu Phe Thr Phe Cys Cys Leu Phe Leu	
200 205 210	
ctg cca ctg act gcc atg gcc atc tgc tat agc cgc att gtg ctc ggt	727
Leu Pro Leu Thr Ala Met Ala Ile Cys Tyr Ser Arg Ile Val Leu Gly	
215 220 225	
gtg tcc agc ccc cgg aca agg aag ggg agc cat gcc cct gcc ggg gaa	775
Val Ser Ser Pro Arg Thr Arg Lys Gly Ser His Ala Pro Ala Gly Glu	
230 235 240 245	
ttt gcc ctc cgt cgc tcc ttc gac aat cgt ccc cgt gtc cgt ctt cgg	823
Phe Ala Leu Arg Arg Ser Phe Asp Asn Arg Pro Arg Val Arg Leu Arg	
250 255 260	
gcc ctg aga ctg gcc ctg ctc gtc ttg ctg acc ttc atc ctc tgc tgg	871
Ala Leu Arg Leu Ala Leu Leu Val Leu Leu Thr Phe Ile Leu Cys Trp	
265 270 275	
aca cct tat tac tta cta ggt ctg tgg tac tgg ttt tcc ccg agc atg	919
Thr Pro Tyr Tyr Leu Leu Gly Leu Trp Tyr Trp Phe Ser Pro Ser Met	
280 285 290	
cta agt gaa gtc cct ccc agc ctc agc cac atc ctt ttc ctc ttt ggc	967
Leu Ser Glu Val Pro Pro Ser Leu Ser His Ile Leu Phe Leu Phe Gly	
295 300 305	
ctc ctc aat gct cct ttg gat cct ctc ctc tat ggg gcc ttc acc ctt	1015
Leu Leu Asn Ala Pro Leu Asp Pro Leu Leu Tyr Gly Ala Phe Thr Leu	
310 315 320 325	
ggc tgc cga aga ggg cac caa gaa ctt agt atg gac tct tct agg gaa	1063
Gly Cys Arg Arg Gly His Gln Glu Leu Ser Met Asp Ser Ser Arg Glu	
330 335 340	
gaa ggg tct agg aga atg ttc caa cag gac att cag gcc ctt aga caa	1111
Glu Gly Ser Arg Arg Met Phe Gln Gln Asp Ile Gln Ala Leu Arg Gln	
345 350 355	
acg gag gta caa aaa act gtg aca tca aga aag gca gga gaa aca aaa	1159
Thr Glu Val Gln Lys Thr Val Thr Ser Arg Lys Ala Gly Glu Thr Lys	
360 365 370	
gac att cct ata aca tca atc tgatcctaac acagtataga gaaacacaat	1210
Asp Ile Pro Ile Thr Ser Ile	
375 380	
aattcttttaa taccataaga tottaacgtc tcaatttcct gctctcctaa toccccccaa	1270
aagaaatact gagcagtgtc tccattttaa cctgcctga acttgagact atgtctaata	1330
cagaaactca cacaactagc ctgggcaaca cagtgaagacc taatctctat agaaatatta	1390
aaagggttaag ccaggcatgg tggcatgtgc ctggaacccc agctactggg aggggtgaggc	1450

agaaggatgg cttgg

1465

<210> 2
 <211> 380
 <212> PRT
 <213> marmoset
 <400> 2

Met Ser Ala Val Asn Gly Thr Pro Trp Gly Ser Ser Ala Arg Glu Glu
 1 5 10 15

Val Trp Ala Gly Ser Gly Val Glu Val Glu Gly Ser Glu Leu Pro Thr
 20 25 30

Phe Ser Thr Ala Ala Lys Val Arg Val Gly Val Thr Ile Val Leu Phe
 35 40 45

Val Ser Ser Ala Gly Gly Asn Leu Ala Val Leu Trp Ser Val Thr Arg
 50 55 60

Pro Gln Pro Ser Gln Leu Arg Pro Ser Pro Val Arg Arg Leu Phe Ala
 65 70 75 80

His Leu Ala Ala Ala Asp Leu Leu Val Thr Phe Val Val Met Pro Leu
 85 90 95

Asp Ala Thr Trp Asn Ile Thr Val Gln Trp Leu Ala Gly Asp Ile Ala
 100 105 110

Cys Arg Thr Leu Met Phe Leu Lys Leu Met Ala Met Tyr Ala Ala Ala
 115 120 125

Phe Leu Pro Val Val Ile Gly Leu Asp Arg Gln Ala Ala Val Leu Asn
 130 135 140

Pro Leu Gly Ser Arg Ser Gly Val Arg Lys Leu Leu Gly Ala Ala Trp
 145 150 155 160

Gly Leu Ser Phe Leu Leu Ala Leu Pro Gln Leu Phe Leu Phe His Thr
 165 170 175

Val His Arg Ala Gly Pro Val Pro Phe Thr Gln Cys Ala Thr Lys Gly
 180 185 190

Ser Phe Lys Ala Arg Trp Gln Glu Thr Thr Tyr Asn Leu Phe Thr Phe
 195 200 205

Cys Cys Leu Phe Leu Leu Pro Leu Thr Ala Met Ala Ile Cys Tyr Ser
 210 215 220
 Arg Ile Val Leu Gly Val Ser Ser Pro Arg Thr Arg Lys Gly Ser His
 225 230 235 240
 Ala Pro Ala Gly Glu Phe Ala Leu Arg Arg Ser Phe Asp Asn Arg Pro
 245 250 255
 Arg Val Arg Leu Arg Ala Leu Arg Leu Ala Leu Leu Val Leu Leu Thr
 260 265 270
 Phe Ile Leu Cys Trp Thr Pro Tyr Tyr Leu Leu Gly Leu Trp Tyr Trp
 275 280 285
 Phe Ser Pro Ser Met Leu Ser Glu Val Pro Pro Ser Leu Ser His Ile
 290 295 300
 Leu Phe Leu Phe Gly Leu Leu Asn Ala Pro Leu Asp Pro Leu Leu Tyr
 305 310 315 320
 Gly Ala Phe Thr Leu Gly Cys Arg Arg Gly His Gln Glu Leu Ser Met
 325 330 335
 Asp Ser Ser Arg Glu Glu Gly Ser Arg Arg Met Phe Gln Gln Asp Ile
 340 345 350
 Gln Ala Leu Arg Gln Thr Glu Val Gln Lys Thr Val Thr Ser Arg Lys
 355 360 365
 Ala Gly Glu Thr Lys Asp Ile Pro Ile Thr Ser Ile
 370 375 380

<210> 3

<211> 2650

<212> DNA

<213> Human

<220><221> misc_feature

<222> (292)..(292)<223> n is absent in the human sequence, but included herein for ease of alignment with the corresponding marmoset sequence

<220><221> misc_feature<222> (292)..(297)<223> 292-297 is a possible intron

<220><221> misc_feature<222> (292)..(300)<223> 291-300 is a possible intron

<220><221> misc_feature<222> (292)..(330)<223> 291-330 is a possible intron

<220><221> misc_feature<222> (798)..(800)<223> 798-800 is the codon tga translated as a selenocysteine (amino acid No. 179)

<220><221> CDS<222> (264)..(1400)<223> The CDS shown includes some apparent amino acids (from Gly 10 onwards) which would be deleted in a short intron, as detailed above.

<400> 3

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cagtagaggc ctgaagccca ggctggtctg tccaaggaaa aaggagcgtg attggtacca      60
gatcttcggt ccctgcagaa ccttgacagt tgaacaagtg acctcctcca gaacagatgg      120
agagtctcca gaagcagagg ctttagtgaa cgaaattcgc aataatcagc tccagatcct      180
gaaaaggagg gcgaagaatc agtggccaaa gctaaccgct tcatacccac acttcatacct      240
cctcagtttc tctccaggcc acc atg tct gca ggc aac ggc acc cct tgg ggn      293
                    Met Ser Ala Gly Asn Gly Thr Pro Trp Gly
                      1             5             10

tca gca gcg ggg gag gag gtc tgg gct gga tca gga gtg gag gtg gag      341
Ser Ala Ala Gly Glu Glu Val Trp Ala Gly Ser Gly Val Glu Val Glu
                      15             20             25

ggc tca gag ctg ccc acc ttc tcg gca gca gcc aag gtc cga gtg gga      389
Gly Ser Glu Leu Pro Thr Phe Ser Ala Ala Ala Lys Val Arg Val Gly
                      30             35             40

gtg acc att gtg ctg ttt gtt tct tcg gct gga ggg aac ctg gca gtc      437
Val Thr Ile Val Leu Phe Val Ser Ser Ala Gly Gly Asn Leu Ala Val
                      45             50             55

ctg tgg tca gtg aca cgg cgg gaa ccc agc cag ctc cgc ccc tgt ccg      485
Leu Trp Ser Val Thr Arg Arg Glu Pro Ser Gln Leu Arg Pro Cys Pro
                      60             65             70

gtc agg aga ctc ttc atc cat tta gca gcc gcc gac tta cta gtc act      533
Val Arg Arg Leu Phe Ile His Leu Ala Ala Ala Asp Leu Leu Val Thr
                      75             80             85             90

ttt gtg gtt atg ccc cta gat gcc acc tgg aat atc act gtt caa tgg      581
Phe Val Val Met Pro Leu Asp Ala Thr Trp Asn Ile Thr Val Gln Trp
                      95             100             105

ctg gct gtg gac atc gca tgt cgg aca ctg atg ttc ctg aaa cta atg      629
Leu Ala Val Asp Ile Ala Cys Arg Thr Leu Met Phe Leu Lys Leu Met
                      110             115             120

gcc acg tat tct gca gct ttc ctg cct gtg gtc att gga ttg gac cgc      677
Ala Thr Tyr Ser Ala Ala Phe Leu Pro Val Val Ile Gly Leu Asp Arg
                      125             130             135

cag gca gca gta ctc aac ccg ctt gga tcc cgt tca ggt gta agg aaa      725
Gln Ala Ala Val Leu Asn Pro Leu Gly Ser Arg Ser Gly Val Arg Lys
                      140             145             150

ctt ctg ggg gca gcc tgg gga ctt agt ttc ctg ctt gcc ttc ccc cag      773
Leu Leu Gly Ala Ala Trp Gly Leu Ser Phe Leu Leu Ala Phe Pro Gln
                      155             160             165             170

ctg ttc ctg ttc cac acg gtc cac nnn gct ggc cca gtc cct ttc act      821

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Leu Phe Leu Phe His Thr Val His Xaa Ala Gly Pro Val Pro Phe Thr	
175 . 180 185	
cag tgt gtc acc aaa ggc agc ttc aag gct caa tgg caa gag acc acc	869
Gln Cys Val Thr Lys Gly Ser Phe Lys Ala Gln Trp Gln Glu Thr Thr	
190 195 200	
tat aac ctc ttc acc ttc tgc tgc ctc ctt ctg ctg cca ctg act gcc	917
Tyr Asn Leu Phe Thr Phe Cys Cys Leu Leu Leu Leu Pro Leu Thr Ala	
205 210 215	
atg gcc atc tgc tat agc cgc att gtc ctc agt gtg tcc agg ccc cag	965
Met Ala Ile Cys Tyr Ser Arg Ile Val Leu Ser Val Ser Arg Pro Gln	
220 225 230	
aca agg aag ggg agc cat gcc cct gct ggt gaa ttt gcc ctc ccc cgc	1013
Thr Arg Lys Gly Ser His Ala Pro Ala Gly Glu Phe Ala Leu Pro Arg	
235 240 245 250	
tcc ttt gac aat tgt ccc cgt gtt cgt ctc cgg gcc ctg aga ctg gcc	1061
Ser Phe Asp Asn Cys Pro Arg Val Arg Leu Arg Ala Leu Arg Leu Ala	
255 260 265	
ctg ctt aac tta ctg acc ttc atc ctc tgc tgg aca cct tat tac cta	1109
Leu Leu Asn Leu Leu Thr Phe Ile Leu Cys Trp Thr Pro Tyr Tyr Leu	
270 275 280	
ctg ggt atg tgg tac tgg ttc tcc ccc acc atg cta act gaa gtc cct	1157
Leu Gly Met Trp Tyr Trp Phe Ser Pro Thr Met Leu Thr Glu Val Pro	
285 290 295	
ccc agc ctg agc cac atc ctt ttc ctc ttg ggc ctc ctc aat gct cct	1205
Pro Ser Leu Ser His Ile Leu Phe Leu Leu Gly Leu Leu Asn Ala Pro	
300 305 310	
ttg gat cct ctc ctc tat ggg gcc ttc acc ctt ggc tgc cga aga ggg	1253
Leu Asp Pro Leu Leu Tyr Gly Ala Phe Thr Leu Gly Cys Arg Arg Gly	
315 320 325 330	
cac caa gaa ctt agt ata gac tct tct aaa gaa ggg tct ggg aga atg	1301
His Gln Glu Leu Ser Ile Asp Ser Ser Lys Glu Gly Ser Gly Arg Met	
335 340 345	
ctc caa gag gag att cat gcc ttt aga cag ctg gaa gta caa aaa act	1349
Leu Gln Glu Glu Ile His Ala Phe Arg Gln Leu Glu Val Gln Lys Thr	
350 355 360	
gtg aca tca aga agg gca gga gaa aca aaa ggc att tct ata aca tct	1397
Val Thr Ser Arg Arg Ala Gly Glu Thr Lys Gly Ile Ser Ile Thr Ser	
365 370 375	
atc tgatcctaac agagtatgta ggaacagaat agtaagtctt tagtgccata	1450
Ile	
agatccttaac atctcacttc tactcctgct ctccctagttc cccccaaaaa agaaatactg	1510
accagtgtct ctactttaaa ccctacctga aacttgagac tatgtctaata atagaaactc	1570
acataactag cccaggtaac acagcaagac cccatctcta caaaaatatt aaaaatttag	1630

ctgggcatgg tggcatgtgc ctgtaatccc aactactagg gacagtgagg cagaaggatg 1690
 gcttgagccc aagagtttga agctgcagtg agctatgac agctgcaatc caccctgggt 1750
 aacacagcaa gactctatct caaaaaaaaag aaaaaaaga aatacataga gttcagtc 1810
 tagaagtatc ttcacaatga tccatacagc cttgctatgc tttagaactt tcaattttag 1870
 gacaggaaag taacattaaa tgtagaaaac aaaaatggaa catttattcg caactcaaat 1930
 actacgcata tacggtaaga gattaaatat aaacacagca agttccaccc cagtcctatt 1990
 tgtccaaggc tgcattgtca aatggaatct tgaagagaac acctggacaa cagaggacct 2050
 gtcagcgacg tctccggtct ggacttctgc tgcgtcttcg gccacctcta gggaaaaaga 2110
 agcagggaga ggagtcatt agagggacat aatactaagt cctcattctg tttcgttcgt 2170
 attcccttca ccagacagt atttgccctc ttcattttac ctctcttgc cttttgggtg 2230
 accccgaaca aaacaccagt caacgctgat gggctgtccc atcaaatact ggccattgag 2290
 tccctccata gcagcctggg ctctcttgta tgtttcatat tcaactagag tataccctg 2350
 gggaaagtga aaagacagat atgaaaaccc tctatttcc ccaagtatca ctgagtatct 2410
 ttttctcaa atctaaactc agaaaaatct acacacttta aacaatgaat gtacatcata 2470
 tatctctact gtatatatac atcatctccg tttctgtctc tttggcgtgt ctgacaaaac 2530
 atagatttcc aatgtcattt tatttcaact ttgctcttgg ccaaccacag caaacacaga 2590
 actacgaatg ctacctaagg cttatgaaag aaaggaggca ataaagtgtc acttaggata 2650

<210> 4

<211> 379

<212> PRT

<213> Human

<220><221> misc_feature

<222> (292)..(292)

<223> n is absent in the human sequence, but included herein for ease of alignment with the corresponding marmoset sequence

<220><221> misc_feature<222> (292)..(297)<223> 292-297 is a possible intron

<220><221> misc_feature<222> (292)..(300)<223> 291-300 is a possible intron

<220><221> misc_feature<222> (292)..(330)<223> 291-330 is a possible intron

<220><221> misc_feature<222> (798)..(800)<223> 798-800 is the codon tga translated as a selenocysteine (amino acid No. 179)

<400> 4

Met Ser Ala Gly Asn Gly Thr Pro Trp Gly Ser Ala Ala Gly Glu Glu
 1 5 10 15

Val Trp Ala Gly Ser Gly Val Glu Val Glu Gly Ser Glu Leu Pro Thr
 20 25 30

Phe Ser Ala Ala Ala Lys Val Arg Val Gly Val Thr Ile Val Leu Phe
 35 40 45

Val Ser Ser Ala Gly Gly Asn Leu Ala Val Leu Trp Ser Val Thr Arg
 50 55 60

Arg Glu Pro Ser Gln Leu Arg Pro Cys Pro Val Arg Arg Leu Phe Ile
 65 70 75 80

His Leu Ala Ala Ala Asp Leu Leu Val Thr Phe Val Val Met Pro Leu
 85 90 95

Asp Ala Thr Trp Asn Ile Thr Val Gln Trp Leu Ala Val Asp Ile Ala
 100 105 110

Cys Arg Thr Leu Met Phe Leu Lys Leu Met Ala Thr Tyr Ser Ala Ala
 115 120 125

Phe Leu Pro Val Val Ile Gly Leu Asp Arg Gln Ala Ala Val Leu Asn
 130 135 140

Pro Leu Gly Ser Arg Ser Gly Val Arg Lys Leu Leu Gly Ala Ala Trp
 145 150 155 160

Gly Leu Ser Phe Leu Leu Ala Phe Pro Gln Leu Phe Leu Phe His Thr
 165 170 175

Val His Xaa Ala Gly Pro Val Pro Phe Thr Gln Cys Val Thr Lys Gly
 180 185 190

Ser Phe Lys Ala Gln Trp Gln Glu Thr Thr Tyr Asn Leu Phe Thr Phe
 195 200 205

Cys Cys Leu Leu Leu Leu Pro Leu Thr Ala Met Ala Ile Cys Tyr Ser
 210 215 220

Arg Ile Val Leu Ser Val Ser Arg Pro Gln Thr Arg Lys Gly Ser His
 225 230 235 240

Ala Pro Ala Gly Glu Phe Ala Leu Pro Arg Ser Phe Asp Asn Cys Pro
 245 250 255

Arg Val Arg Leu Arg Ala Leu Arg Leu Ala Leu Leu Asn Leu Leu Thr
 260 265 270

Phe Ile Leu Cys Trp Thr Pro Tyr Tyr Leu Leu Gly Met Trp Tyr Trp
 275 280 285

Phe Ser Pro Thr Met Leu Thr Glu Val Pro Pro Ser Leu Ser His Ile
 290 295 300

Leu Phe Leu Leu Gly Leu Leu Asn Ala Pro Leu Asp Pro Leu Leu Tyr
 305 310 315 320

Gly Ala Phe Thr Leu Gly Cys Arg Arg Gly His Gln Glu Leu Ser Ile
 325 330 335

Asp Ser Ser Lys Glu Gly Ser Gly Arg Met Leu Gln Glu Glu Ile His
 340 345 350

Ala Phe Arg Gln Leu Glu Val Gln Lys Thr Val Thr Ser Arg Arg Ala
 355 360 365

Gly Glu Thr Lys Gly Ile Ser Ile Thr Ser Ile
 370 375

<210> 5

<211> 1397

<212> DNA

<213> Human

<220><221> CDS

<222> (264)..(1394)

<220><221> misc_feature

<222> (792)..(794)<223> 792-794 is the codon tga translated as
 selenocysteine (amino acid No. 177)

<400> 5

cagtagaggc ctgaagccca ggctggtctg tccaaggaaa aaggagcgtg attggtacca 60

gatcttcgtt ccttcagaaa ccttgacagt tgaacaagtg acctcctcca gaacagatgg 120

agagtctcca gaagcagagg ctttagtgaa cgaaattcgc aataatcagc tccagatcct 180

gaaaaggagg gcgaagaatc agtggccaaa gctaaccgct tcatacccac acttcaccc 240

cctcagtttc tctccaggcc acc atg tct gca ggc aac ggc acc cct tgg gca 293

Met Ser Ala Gly Asn Gly Thr Pro Trp Ala
 1 5 10

gcg ggg gag gag gtc tgg gct gga tca gga gtg gag gtg gag ggc tca 341

Ala Gly Glu Glu Val Trp Ala Gly Ser Gly Val Glu Val Glu Gly Ser
 15 20 25

gag ctg ccc acc ttc tcg gca gca gcc aag gtc cga gtg gga gtg acc 389

Glu Leu Pro Thr Phe Ser Ala Ala Ala Lys Val Arg Val Gly Val Thr

30										35										40									
att	gtg	ctg	ttt	gtt	tct	tcg	gct	gga	ggg	aac	ctg	gca	gtc	ctg	tgg														
Ile	Val	Leu	Phe	Val	Ser	Ser	Ala	Gly	Gly	Asn	Leu	Ala	Val	Leu	Trp														
		45						50				55																	
tca	gtg	aca	cgg	cgg	gaa	ccc	agc	cag	ctc	cgc	ccc	tgt	ccg	gtc	agg														
Ser	Val	Thr	Arg	Arg	Glu	Pro	Ser	Gln	Leu	Arg	Pro	Cys	Pro	Val	Arg														
		60				65				70																			
aga	ctc	ttc	atc	cat	tta	gca	gcc	gcc	gac	tta	cta	gtc	act	ttt	gtg														
Arg	Leu	Phe	Ile	His	Leu	Ala	Ala	Ala	Asp	Leu	Leu	Val	Thr	Phe	Val														
		75			80					85					90														
gtt	atg	ccc	cta	gat	gcc	acc	tgg	aat	atc	act	gtt	caa	tgg	ctg	gct														
Val	Met	Pro	Leu	Asp	Ala	Thr	Trp	Asn	Ile	Thr	Val	Gln	Trp	Leu	Ala														
				95				100						105															
gtg	gac	atc	gca	tgt	cgg	aca	ctg	atg	ttc	ctg	aaa	cta	atg	gcc	acg														
Val	Asp	Ile	Ala	Cys	Arg	Thr	Leu	Met	Phe	Leu	Lys	Leu	Met	Ala	Thr														
			110					115					120																
tat	tct	gca	gct	ttc	ctg	cct	gtg	gtc	att	gga	ttg	gac	cgc	cag	gca														
Tyr	Ser	Ala	Ala	Phe	Leu	Pro	Val	Val	Ile	Gly	Leu	Asp	Arg	Gln	Ala														
		125					130					135																	
gca	gta	ctc	aac	ccg	ctt	gga	tcc	cgt	tca	ggt	gta	agg	aaa	ctt	ctg														
Ala	Val	Leu	Asn	Pro	Leu	Gly	Ser	Arg	Ser	Gly	Val	Arg	Lys	Leu	Leu														
		140				145					150																		
ggg	gca	gcc	tgg	gga	ctt	agt	ttc	ctg	ctt	gcc	ttc	ccc	cag	ctg	ttc														
Gly	Ala	Ala	Trp	Gly	Leu	Ser	Phe	Leu	Leu	Ala	Phe	Pro	Gln	Leu	Phe														
		155			160					165				170															
ctg	ttc	cac	acg	gtc	cac	nnn	gct	ggc	cca	gtc	cct	ttc	act	cag	tgt														
Leu	Phe	His	Thr	Val	His	Xaa	Ala	Gly	Pro	Val	Pro	Phe	Thr	Gln	Cys														
				175				180						185															
gtc	acc	aaa	ggc	agc	ttc	aag	gct	caa	tgg	caa	gag	acc	acc	tat	aac														
Val	Thr	Lys	Gly	Ser	Phe	Lys	Ala	Gln	Trp	Gln	Glu	Thr	Thr	Tyr	Asn														
			190					195				200																	
ctc	ttc	acc	ttc	tgc	tgc	ctc	ctt	ctg	ctg	cca	ctg	act	gcc	atg	gcc														
Leu	Phe	Thr	Phe	Cys	Cys	Leu	Leu	Leu	Leu	Pro	Leu	Thr	Ala	Met	Ala														
			205				210					215																	
atc	tgc	tat	agc	cgc	att	gtc	ctc	agt	gtg	tcc	agg	ccc	cag	aca	agg														
Ile	Cys	Tyr	Ser	Arg	Ile	Val	Leu	Ser	Val	Ser	Arg	Pro	Gln	Thr	Arg														
		220				225					230																		
aag	ggg	agc	cat	gcc	cct	gct	ggt	gaa	ttt	gcc	ctc	ccc	cgc	tcc	ttt														
Lys	Gly	Ser	His	Ala	Pro	Ala	Gly	Glu	Phe	Ala	Leu	Pro	Arg	Ser	Phe														
		235			240					245				250															
gac	aat	tgt	ccc	cgt	gtt	cgt	ctc	cgg	gcc	ctg	aga	ctg	gcc	ctg	ctt														
Asp	Asn	Cys	Pro	Arg	Val	Arg	Leu	Arg	Ala	Leu	Arg	Leu	Ala	Leu	Leu														
				255				260					265																
aac	tta	ctg	acc	ttc	atc	ctc	tgc	tgg	aca	cct	tat	tac	cta	ctg	ggt														
Asn	Leu	Leu	Thr	Phe	Ile	Leu	Cys	Trp	Thr	Pro	Tyr	Tyr	Leu	Leu	Gly														
			270				275						280																

atg tgg tac tgg ttc tcc ccc acc atg cta act gaa gtc cct ccc agc 1157
 Met Trp Tyr Trp Phe Ser Pro Thr Met Leu Thr Glu Val Pro Pro Ser
 285 290 295

ctg agc cac atc ctt ttc ctc ttg ggc ctc ctc aat gct cct ttg gat 1205
 Leu Ser His Ile Leu Phe Leu Leu Gly Leu Leu Asn Ala Pro Leu Asp
 300 305 310

cct ctc ctc tat ggg gcc ttc acc ctt ggc tgc cga aga ggg cac caa 1253
 Pro Leu Leu Tyr Gly Ala Phe Thr Leu Gly Cys Arg Arg Gly His Gln
 315 320 325 330

gaa ctt agt ata gac tct tct aaa gaa ggg tct ggg aga atg ctc caa 1301
 Glu Leu Ser Ile Asp Ser Ser Lys Glu Gly Ser Gly Arg Met Leu Gln
 335 340 345

gag gag att cat gcc ttt aga cag ctg gaa gta caa aaa act gtg aca 1349
 Glu Glu Ile His Ala Phe Arg Gln Leu Glu Val Gln Lys Thr Val Thr
 350 355 360

tca aga agg gca gga gaa aca aaa ggc att tct ata aca tct atc tga 1397
 Ser Arg Arg Ala Gly Glu Thr Lys Gly Ile Ser Ile Thr Ser Ile
 365 370 375

<210> 6

<211> 377

<212> PRT

<213> Human

<220><221> misc_feature

<222> (792)..(794)<223> 792-794 is the codon tga translated as
 selenocysteine (amino acid No. 177)

<400> 6

Met Ser Ala Gly Asn Gly Thr Pro Trp Ala Ala Gly Glu Glu Val Trp
 1 5 10 15

Ala Gly Ser Gly Val Glu Val Glu Gly Ser Glu Leu Pro Thr Phe Ser
 20 25 30

Ala Ala Ala Lys Val Arg Val Gly Val Thr Ile Val Leu Phe Val Ser
 35 40 45

Ser Ala Gly Gly Asn Leu Ala Val Leu Trp Ser Val Thr Arg Arg Glu
 50 55 60

Pro Ser Gln Leu Arg Pro Cys Pro Val Arg Arg Leu Phe Ile His Leu
 65 70 75 80

Ala Ala Ala Asp Leu Leu Val Thr Phe Val Val Met Pro Leu Asp Ala
 85 90 95

Thr Trp Asn Ile Thr Val Gln Trp Leu Ala Val Asp Ile Ala Cys Arg

100					105					110					
Thr	Leu	Met	Phe	Leu	Lys	Leu	Met	Ala	Thr	Tyr	Ser	Ala	Ala	Phe	Leu
	115						120					125			
Pro	Val	Val	Ile	Gly	Leu	Asp	Arg	Gln	Ala	Ala	Val	Leu	Asn	Pro	Leu
	130					135					140				
Gly	Ser	Arg	Ser	Gly	Val	Arg	Lys	Leu	Leu	Gly	Ala	Ala	Trp	Gly	Leu
145						150					155				160
Ser	Phe	Leu	Leu	Ala	Phe	Pro	Gln	Leu	Phe	Leu	Phe	His	Thr	Val	His
				165					170					175	
Xaa	Ala	Gly	Pro	Val	Pro	Phe	Thr	Gln	Cys	Val	Thr	Lys	Gly	Ser	Phe
			180					185					190		
Lys	Ala	Gln	Trp	Gln	Glu	Thr	Thr	Tyr	Asn	Leu	Phe	Thr	Phe	Cys	Cys
		195					200					205			
Leu	Leu	Leu	Leu	Pro	Leu	Thr	Ala	Met	Ala	Ile	Cys	Tyr	Ser	Arg	Ile
	210					215					220				
Val	Leu	Ser	Val	Ser	Arg	Pro	Gln	Thr	Arg	Lys	Gly	Ser	His	Ala	Pro
225						230					235				240
Ala	Gly	Glu	Phe	Ala	Leu	Pro	Arg	Ser	Phe	Asp	Asn	Cys	Pro	Arg	Val
				245					250					255	
Arg	Leu	Arg	Ala	Leu	Arg	Leu	Ala	Leu	Leu	Asn	Leu	Leu	Thr	Phe	Ile
			260					265					270		
Leu	Cys	Trp	Thr	Pro	Tyr	Tyr	Leu	Leu	Gly	Met	Trp	Tyr	Trp	Phe	Ser
		275					280					285			
Pro	Thr	Met	Leu	Thr	Glu	Val	Pro	Pro	Ser	Leu	Ser	His	Ile	Leu	Phe
	290					295					300				
Leu	Leu	Gly	Leu	Leu	Asn	Ala	Pro	Leu	Asp	Pro	Leu	Leu	Tyr	Gly	Ala
305						310					315				320
Phe	Thr	Leu	Gly	Cys	Arg	Arg	Gly	His	Gln	Glu	Leu	Ser	Ile	Asp	Ser
				325					330					335	
Ser	Lys	Glu	Gly	Ser	Gly	Arg	Met	Leu	Gln	Glu	Glu	Ile	His	Ala	Phe
		340						345					350		

Arg Gln Leu Glu Val Gln Lys Thr Val Thr Ser Arg Arg Ala Gly Glu
 355 360 365

Thr Lys Gly Ile Ser Ile Thr Ser Ile
 370 375

<210> 7
 <211> 1394
 <212> DNA
 <213> human
 <220><221> CDS
 <222> (264)..(1391)
 <220><221> misc_feature<222> (789)..(791)<223> codon 789-791 is the
 codon tga translated as a selenocysteine (amino acid No. 176)

<400> 7
 cagtagaggc ctgaagccca ggctgggtctg tccaaggaaa aaggagcgtg attggtacca 60
 gatcttcggt cccctgcagaa ccttgacagt tgaacaagtg acctcctcca gaacagatgg 120
 agagtctcca gaagcagagg ctttagtgaa cgaaattcgc aataatcagc tccagatcct 180
 gaaaaggagg gcgaagaatc agtggccaaa gctaaccgct tcatacccac acttcatcct 240
 cctcagtttc tctccaggcc acc atg tct gca ggc aac ggc acc cct tgg gcg 293
 Met Ser Ala Gly Asn Gly Thr Pro Trp Ala
 1 5 10
 ggg gag gag gtc tgg gct gga tca gga gtg gag gtg gag ggc tca gag 341
 Gly Glu Glu Val Trp Ala Gly Ser Gly Val Glu Val Glu Gly Ser Glu
 15 20 25
 ctg ccc acc ttc tcg gca gca gcc aag gtc cga gtg gga gtg acc att 389
 Leu Pro Thr Phe Ser Ala Ala Ala Lys Val Arg Val Gly Val Thr Ile
 30 35 40
 gtg ctg ttt gtt tct tcg gct gga ggg aac ctg gca gtc ctg tgg tca 437
 Val Leu Phe Val Ser Ser Ala Gly Gly Asn Leu Ala Val Leu Trp Ser
 45 50 55
 gtg aca cgg cgg gaa ccc agc cag ctg cgc ccc tgt ccg gtc agg aga 485
 Val Thr Arg Arg Glu Pro Ser Gln Leu Arg Pro Cys Pro Val Arg Arg
 60 65 70
 ctc ttc atc cat tta gca gcc gcc gac tta cta gtc act ttt gtg gtt 533
 Leu Phe Ile His Leu Ala Ala Ala Asp Leu Leu Val Thr Phe Val Val
 75 80 85 90
 atg ccc cta gat gcc acc tgg aat atc act gtt caa tgg ctg gct gtg 581
 Met Pro Leu Asp Ala Thr Trp Asn Ile Thr Val Gln Trp Leu Ala Val
 95 100 105
 gac atc gca tgt cgg aca ctg atg ttc ctg aaa cta atg gcc acg tat 629
 Asp Ile Ala Cys Arg Thr Leu Met Phe Leu Lys Leu Met Ala Thr Tyr
 110 115 120
 tct gca gct ttc ctg cct gtg gtc att gga ttg gac cgc cag gca gca 677

Ser	Ala	Ala	Phe	Leu	Pro	Val	Val	Ile	Gly	Leu	Asp	Arg	Gln	Ala	Ala		
	125						130					135					
gta	ctc	aac	ccg	ctt	gga	tcc	cgt	tca	ggc	gta	agg	aaa	ctt	ctg	ggg		725
Val	Leu	Asn	Pro	Leu	Gly	Ser	Arg	Ser	Gly	Val	Arg	Lys	Leu	Leu	Gly		
	140					145					150						
gca	gcc	tgg	gga	ctt	agt	ttc	ctg	ctt	gcc	ttc	ccc	cag	ctg	ttc	ctg		773
Ala	Ala	Trp	Gly	Leu	Ser	Phe	Leu	Leu	Ala	Phe	Pro	Gln	Leu	Phe	Leu		
	155				160					165					170		
ttc	cac	acg	gtc	cac	nnn	gct	ggc	cca	gtc	cct	ttc	act	cag	tgt	gtc		821
Phe	His	Thr	Val	His	Xaa	Ala	Gly	Pro	Val	Pro	Phe	Thr	Gln	Cys	Val		
				175					180					185			
acc	aaa	ggc	agc	ttc	aag	gct	caa	tgg	caa	gag	acc	acc	tat	aac	ctc		869
Thr	Lys	Gly	Ser	Phe	Lys	Ala	Gln	Trp	Gln	Glu	Thr	Thr	Tyr	Asn	Leu		
			190					195					200				
ttc	acc	ttc	tgc	tgc	ctc	ctt	ctg	ctg	cca	ctg	act	gcc	atg	gcc	atc		917
Phe	Thr	Phe	Cys	Cys	Leu	Leu	Leu	Leu	Pro	Leu	Thr	Ala	Met	Ala	Ile		
		205					210					215					
tgc	tat	agc	cgc	att	gtc	ctc	agt	gtg	tcc	agg	ccc	cag	aca	agg	aag		965
Cys	Tyr	Ser	Arg	Ile	Val	Leu	Ser	Val	Ser	Arg	Pro	Gln	Thr	Arg	Lys		
	220					225					230						
ggg	agc	cat	gcc	cct	gct	ggc	gaa	ttt	gcc	ctc	ccc	cgc	tcc	ttt	gac		1013
Gly	Ser	His	Ala	Pro	Ala	Gly	Glu	Phe	Ala	Leu	Pro	Arg	Ser	Phe	Asp		
	235				240					245					250		
aat	tgt	ccc	cgt	gtt	cgt	ctc	cgg	gcc	ctg	aga	ctg	gcc	ctg	ctt	aac		1061
Asn	Cys	Pro	Arg	Val	Arg	Leu	Arg	Ala	Leu	Arg	Leu	Ala	Leu	Leu	Asn		
				255					260					265			
tta	ctg	acc	ttc	atc	ctc	tgc	tgg	aca	cct	tat	tac	cta	ctg	ggc	atg		1109
Leu	Leu	Thr	Phe	Ile	Leu	Cys	Trp	Thr	Pro	Tyr	Tyr	Leu	Leu	Gly	Met		
			270					275					280				
tgg	tac	tgg	ttc	tcc	ccc	acc	atg	cta	act	gaa	gtc	cct	ccc	agc	ctg		1157
Trp	Tyr	Trp	Phe	Ser	Pro	Thr	Met	Leu	Thr	Glu	Val	Pro	Pro	Ser	Leu		
		285					290					295					
agc	cac	atc	ctt	ttc	ctc	ttg	ggc	ctc	ctc	aat	gct	cct	ttg	gat	cct		1205
Ser	His	Ile	Leu	Phe	Leu	Leu	Gly	Leu	Leu	Asn	Ala	Pro	Leu	Asp	Pro		
	300					305				310							
ctc	ctc	tat	ggg	gcc	ttc	acc	ctt	ggc	tgc	cga	aga	ggg	cac	caa	gaa		1253
Leu	Leu	Tyr	Gly	Ala	Phe	Thr	Leu	Gly	Cys	Arg	Arg	Gly	His	Gln	Glu		
	315				320					325					330		
ctt	agt	ata	gac	tct	tct	aaa	gaa	ggg	tct	ggg	aga	atg	ctc	caa	gag		1301
Leu	Ser	Ile	Asp	Ser	Ser	Lys	Glu	Gly	Ser	Gly	Arg	Met	Leu	Gln	Glu		
				335					340					345			
gag	att	cat	gcc	ttt	aga	cag	ctg	gaa	gta	caa	aaa	act	gtg	aca	tca		1349
Glu	Ile	His	Ala	Phe	Arg	Gln	Leu	Glu	Val	Gln	Lys	Thr	Val	Thr	Ser		
			350					355					360				
aga	agg	gca	gga	gaa	aca	aaa	ggc	att	tct	ata	aca	tct	atc	tga			1394
Arg	Arg	Ala	Gly	Glu	Thr	Lys	Gly	Ile	Ser	Ile	Thr	Ser	Ile				

365

370

375

<210> 8

<211> 376

<212> PRT

<213> human

<220><221> misc_feature

<222> (789)..(791)

<223> codon 789-791 is the codon tga translated as a selenocysteine (amino acid No. 176)

<400> 8

Met Ser Ala Gly Asn Gly Thr Pro Trp Ala Gly Glu Glu Val Trp Ala
 1 5 10 15

Gly Ser Gly Val Glu Val Glu Gly Ser Glu Leu Pro Thr Phe Ser Ala
 20 25 30

Ala Ala Lys Val Arg Val Gly Val Thr Ile Val Leu Phe Val Ser Ser
 35 40 45

Ala Gly Gly Asn Leu Ala Val Leu Trp Ser Val Thr Arg Arg Glu Pro
 50 55 60

Ser Gln Leu Arg Pro Cys Pro Val Arg Arg Leu Phe Ile His Leu Ala
 65 70 75 80

Ala Ala Asp Leu Leu Val Thr Phe Val Val Met Pro Leu Asp Ala Thr
 85 90 95

Trp Asn Ile Thr Val Gln Trp Leu Ala Val Asp Ile Ala Cys Arg Thr
 100 105 110

Leu Met Phe Leu Lys Leu Met Ala Thr Tyr Ser Ala Ala Phe Leu Pro
 115 120 125

Val Val Ile Gly Leu Asp Arg Gln Ala Ala Val Leu Asn Pro Leu Gly
 130 135 140

Ser Arg Ser Gly Val Arg Lys Leu Leu Gly Ala Ala Trp Gly Leu Ser
 145 150 155 160

Phe Leu Leu Ala Phe Pro Gln Leu Phe Leu Phe His Thr Val His Xaa
 165 170 175

Ala Gly Pro Val Pro Phe Thr Gln Cys Val Thr Lys Gly Ser Phe Lys
 180 185 190

Ala Gln Trp Gln Glu Thr Thr Tyr Asn Leu Phe Thr Phe Cys Cys Leu
 195 200 205

Leu Leu Leu Pro Leu Thr Ala Met Ala Ile Cys Tyr Ser Arg Ile Val
 210 215 220

Leu Ser Val Ser Arg Pro Gln Thr Arg Lys Gly Ser His Ala Pro Ala
 225 230 235 240

Gly Glu Phe Ala Leu Pro Arg Ser Phe Asp Asn Cys Pro Arg Val Arg
 245 250 255

Leu Arg Ala Leu Arg Leu Ala Leu Leu Asn Leu Leu Thr Phe Ile Leu
 260 265 270

Cys Trp Thr Pro Tyr Tyr Leu Leu Gly Met Trp Tyr Trp Phe Ser Pro
 275 280 285

Thr Met Leu Thr Glu Val Pro Pro Ser Leu Ser His Ile Leu Phe Leu
 290 295 300

Leu Gly Leu Leu Asn Ala Pro Leu Asp Pro Leu Leu Tyr Gly Ala Phe
 305 310 315 320

Thr Leu Gly Cys Arg Arg Gly His Gln Glu Leu Ser Ile Asp Ser Ser
 325 330 335

Lys Glu Gly Ser Gly Arg Met Leu Gln Glu Glu Ile His Ala Phe Arg
 340 345 350

Gln Leu Glu Val Gln Lys Thr Val Thr Ser Arg Arg Ala Gly Glu Thr
 355 360 365

Lys Gly Ile Ser Ile Thr Ser Ile
 370 375

<210> 9

<211> 1364

<212> DNA

<213> human

<220><221> CDS

<222> (264)..(1361)

<220><221> misc_feature<222> (759)..(761)<223> codon 759-761 is the
 codon tga translated as selenocysteine (amino acid 166)

<400> 9

cagtagagggc ctgaagccca ggctgggtctg tccaaggaaa aaggagcgtg attggtacca 60

gatcttcggtt cctgcagaa ccttgacagt tgaacaagtg acctcctcca gaacagatgg	120
agagtctcca gaagcagagg ctttagtgaa cgaaattcgc aataatcagc tccagatcct	180
gaaaaggagg gcgaagaatc agtggccaaa gctaaccgct tcataccac acttcacct	240
cctcagtttc tctccaggcc acc atg tct gca ggc aac ggc acc cct tgg gtg	293
Met Ser Ala Gly Asn Gly Thr Pro Trp Val	
1 5 10	
gag gtg gag ggc tca gag ctg ccc acc ttc tcg gca gca gcc aag gtc	341
Glu Val Glu Gly Ser Glu Leu Pro Thr Phe Ser Ala Ala Ala Lys Val	
15 20 25	
cga gtg gga gtg acc att gtg ctg ttt gtt tct tcg gct gga ggg aac	389
Arg Val Gly Val Thr Ile Val Leu Phe Val Ser Ser Ala Gly Gly Asn	
30 35 40	
ctg gca gtc ctg tgg tca gtg aca cgg cgg gaa ccc agc cag ctc cgc	437
Leu Ala Val Leu Trp Ser Val Thr Arg Arg Glu Pro Ser Gln Leu Arg	
45 50 55	
ccc tgt ccg gtc agg aga ctc ttc atc cat tta gca gcc gcc gac tta	485
Pro Cys Pro Val Arg Arg Leu Phe Ile His Leu Ala Ala Ala Asp Leu	
60 65 70	
cta gtc act ttt gtg gtt atg ccc cta gat gcc acc tgg aat atc act	533
Leu Val Thr Phe Val Val Met Pro Leu Asp Ala Thr Trp Asn Ile Thr	
75 80 85 90	
gtt caa tgg ctg gct gtg gac atc gca tgt cgg aca ctg atg ttc ctg	581
Val Gln Trp Leu Ala Val Asp Ile Ala Cys Arg Thr Leu Met Phe Leu	
95 100 105	
aaa cta atg gcc acg tat tct gca gct ttc ctg cct gtg gtc att gga	629
Lys Leu Met Ala Thr Tyr Ser Ala Ala Phe Leu Pro Val Val Ile Gly	
110 115 120	
ttg gac cgc cag gca gca gta ctc aac ccg ctt gga tcc cgt tca ggt	677
Leu Asp Arg Gln Ala Ala Val Leu Asn Pro Leu Gly Ser Arg Ser Gly	
125 130 135	
gta agg aaa ctt ctg ggg gca gcc tgg gga ctt agt ttc ctg ctt gcc	725
Val Arg Lys Leu Leu Gly Ala Ala Trp Gly Leu Ser Phe Leu Leu Ala	
140 145 150	
ttc ccc cag ctg ttc ctg ttc cac acg gtc cac nnn gct ggc cca gtc	773
Phe Pro Gln Leu Phe Leu Phe His Thr Val His Xaa Ala Gly Pro Val	
155 160 165 170	
cct ttc act cag tgt gtc acc aaa ggc agc ttc aag gct caa tgg caa	821
Pro Phe Thr Gln Cys Val Thr Lys Gly Ser Phe Lys Ala Gln Trp Gln	
175 180 185	
gag acc acc tat aac ctc ttc acc ttc tgc tgc ctc ctt ctg ctg cca	869
Glu Thr Thr Tyr Asn Leu Phe Thr Phe Cys Cys Leu Leu Leu Leu Pro	
190 195 200	
ctg act gcc atg gcc atc tgc tat agc cgc att gtc ctc agt gtg tcc	917
Leu Thr Ala Met Ala Ile Cys Tyr Ser Arg Ile Val Leu Ser Val Ser	
205 210 215	

agg ccc cag aca agg aag ggg agc cat gcc cct gct ggt gaa ttt gcc 965
 Arg Pro Gln Thr Arg Lys Gly Ser His Ala Pro Ala Gly Glu Phe Ala
 220 225 230

ctc ccc cgc tcc ttt gac aat tgt ccc cgt gtt cgt ctc cgg gcc ctg 1013
 Leu Pro Arg Ser Phe Asp Asn Cys Pro Arg Val Arg Leu Arg Ala Leu
 235 240 245 250

aga ctg gcc ctg ctt aac tta ctg acc ttc atc ctc tgc tgg aca cct 1061
 Arg Leu Ala Leu Leu Asn Leu Leu Thr Phe Ile Leu Cys Trp Thr Pro
 255 260 265

tat tac cta ctg ggt atg tgg tac tgg ttc tcc ccc acc atg cta act 1109
 Tyr Tyr Leu Leu Gly Met Trp Tyr Trp Phe Ser Pro Thr Met Leu Thr
 270 275 280

gaa gtc cct ccc agc ctg agc cac atc ctt ttc ctc ttg ggc ctc ctc 1157
 Glu Val Pro Pro Ser Leu Ser His Ile Leu Phe Leu Leu Gly Leu Leu
 285 290 295

aat gct cct ttg gat cct ctc ctc tat ggg gcc ttc acc ctt ggc tgc 1205
 Asn Ala Pro Leu Asp Pro Leu Leu Tyr Gly Ala Phe Thr Leu Gly Cys
 300 305 310

cga aga ggg cac caa gaa ctt agt ata gac tct tct aaa gaa ggg tct 1253
 Arg Arg Gly His Gln Glu Leu Ser Ile Asp Ser Ser Lys Glu Gly Ser
 315 320 325 330

ggg aga atg ctc caa gag gag att cat gcc ttt aga cag ctg gaa gta 1301
 Gly Arg Met Leu Gln Glu Glu Ile His Ala Phe Arg Gln Leu Glu Val
 335 340 345

caa aaa act gtg aca tca aga agg gca gga gaa aca aaa ggc att tct 1349
 Gln Lys Thr Val Thr Ser Arg Arg Ala Gly Glu Thr Lys Gly Ile Ser
 350 355 360

ata aca tct atc tga 1364
 Ile Thr Ser Ile
 365

<210> 10

<211> 366

<212> PRT

<213> human

<220><221> misc_feature

<222> (759)..(761)<223> codon 759-761 is the codon tga translated as
 selenocysteine (amino acid 166)

<400> 10

Met Ser Ala Gly Asn Gly Thr Pro Trp Val Glu Val Glu Gly Ser Glu
 1 5 10 15

Leu Pro Thr Phe Ser Ala Ala Ala Lys Val Arg Val Gly Val Thr Ile
 20 25 30

Val Leu Phe Val Ser Ser Ala Gly Gly Asn Leu Ala Val Leu Trp Ser

35					40					45									
Val	Thr	Arg	Arg	Glu	Pro	Ser	Gln	Leu	Arg	Pro	Cys	Pro	Val	Arg	Arg				
50					55					60									
Leu	Phe	Ile	His	Leu	Ala	Ala	Ala	Asp	Leu	Leu	Val	Thr	Phe	Val	Val				
65					70					75					80				
Met	Pro	Leu	Asp	Ala	Thr	Trp	Asn	Ile	Thr	Val	Gln	Trp	Leu	Ala	Val				
85					90					95									
Asp	Ile	Ala	Cys	Arg	Thr	Leu	Met	Phe	Leu	Lys	Leu	Met	Ala	Thr	Tyr				
100					105					110									
Ser	Ala	Ala	Phe	Leu	Pro	Val	Val	Ile	Gly	Leu	Asp	Arg	Gln	Ala	Ala				
115					120					125									
Val	Leu	Asn	Pro	Leu	Gly	Ser	Arg	Ser	Gly	Val	Arg	Lys	Leu	Leu	Gly				
130					135					140									
Ala	Ala	Trp	Gly	Leu	Ser	Phe	Leu	Leu	Ala	Phe	Pro	Gln	Leu	Phe	Leu				
145					150					155					160				
Phe	His	Thr	Val	His	Xaa	Ala	Gly	Pro	Val	Pro	Phe	Thr	Gln	Cys	Val				
165					170					175									
Thr	Lys	Gly	Ser	Phe	Lys	Ala	Gln	Trp	Gln	Glu	Thr	Thr	Tyr	Asn	Leu				
180					185					190									
Phe	Thr	Phe	Cys	Cys	Leu	Leu	Leu	Leu	Pro	Leu	Thr	Ala	Met	Ala	Ile				
195					200					205									
Cys	Tyr	Ser	Arg	Ile	Val	Leu	Ser	Val	Ser	Arg	Pro	Gln	Thr	Arg	Lys				
210					215					220									
Gly	Ser	His	Ala	Pro	Ala	Gly	Glu	Phe	Ala	Leu	Pro	Arg	Ser	Phe	Asp				
225					230					235					240				
Asn	Cys	Pro	Arg	Val	Arg	Leu	Arg	Ala	Leu	Arg	Leu	Ala	Leu	Leu	Asn				
245					250					255									
Leu	Leu	Thr	Phe	Ile	Leu	Cys	Trp	Thr	Pro	Tyr	Tyr	Leu	Leu	Gly	Met				
260					265					270									
Trp	Tyr	Trp	Phe	Ser	Pro	Thr	Met	Leu	Thr	Glu	Val	Pro	Pro	Ser	Leu				
275					280					285									

Ser His Ile Leu Phe Leu Leu Gly Leu Leu Asn Ala Pro Leu Asp Pro
 290 295 300

Leu Leu Tyr Gly Ala Phe Thr Leu Gly Cys Arg Arg Gly His Gln Glu
 305 310 315 320

Leu Ser Ile Asp Ser Ser Lys Glu Gly Ser Gly Arg Met Leu Gln Glu
 325 330 335

Glu Ile His Ala Phe Arg Gln Leu Glu Val Gln Lys Thr Val Thr Ser
 340 345 350

Arg Arg Ala Gly Glu Thr Lys Gly Ile Ser Ile Thr Ser Ile
 355 360 365

<210> 11

<211> 51

<212> DNA

<213> human

<220><221> CDS

<222> (1)..(51)

<400> 11

atg tct gca ggc aac ggc acc cct tgg gca gcg ggg gag gag gtc tgg 48
 Met Ser Ala Gly Asn Gly Thr Pro Trp Ala Ala Gly Glu Glu Val Trp
 1 5 10 15

gct 51
 Ala

<210> 12

<211> 17

<212> PRT

<213> human

<400> 12

Met Ser Ala Gly Asn Gly Thr Pro Trp Ala Ala Gly Glu Glu Val Trp
 1 5 10 15

Ala

<210> 13

<211> 51

<212> DNA

<213> human

<220><221> CDS

<222> (1)..(51)

<400> 13

atg tct gca ggc aac ggc acc cct tgg gcg ggg gag gag gtc tgg gct 48

Met Ser Ala Gly Asn Gly Thr Pro Trp Ala Gly Glu Glu Val Trp Ala
 1 5 10 15

gga
 Gly

51

<210> 14
 <211> 17
 <212> PRT
 <213> human
 <400> 14

Met Ser Ala Gly Asn Gly Thr Pro Trp Ala Gly Glu Glu Val Trp Ala
 1 5 10 15

Gly

<210> 15
 <211> 51
 <212> DNA
 <213> human
 <220><221> CDS
 <222> (1)..(51)
 <400> 15

atg tct gca ggc aac ggc acc cct tgg gtg gag gtg gag ggc tca gag
 Met Ser Ala Gly Asn Gly Thr Pro Trp Val Glu Val Glu Gly Ser Glu
 1 5 10 15

48

ctg
 Leu

51

<210> 16
 <211> 17
 <212> PRT
 <213> human
 <400> 16

Met Ser Ala Gly Asn Gly Thr Pro Trp Val Glu Val Glu Gly Ser Glu
 1 5 10 15

Leu

<210> 17
 <211> 22
 <212> DNA
 <213> Synthetic primers
 <400> 17
 gatgccacct ggaatatcac tg

22

<210> 18
 <211> 13
 <212> DNA
 <213> Synthetic primers

<400> 18	
aggcagcaga agg	13
<210> 19	
<211> 23	
<212> DNA	
<213> Synthetic primer	
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cagcctgggg acttagtttc ctg	23
<210> 20	
<211> 20	
<212> DNA	
<213> Synthetic Primers	
<400> 20	
ggttataggt ggtctcttgc	20
<210> 21	
<211> 39	
<212> DNA	
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gaccacgcgt atcgatgtcg actttttttt ttttttttv	39
<210> 22	
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<212> DNA	
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gaagggactg gaccagctcg	20
<210> 23	
<211> 22	
<212> DNA	
<213> Synthetic Primer	
<400> 23	
gaccacgcgt atcgatgtcg ac	22
<210> 24	
<211> 21	
<212> DNA	
<213> Synthetic Primers	
<400> 24	
caaggcaagc aggaaactaa g	21
<210> 25	
<211> 22	
<212> DNA	
<213> Synthetic Primers	
<400> 25	
acctcttcac cttctgctgc ct	22
<210> 26	

<211> 23
 <212> DNA
 <213> Synthetic Primers
 <400> 26
 cctcctcaat gtcctttgg atc 23

<210> 27
 <211> 27
 <212> DNA
 <213> Synthetic Primer
 <400> 27
 gaattcgctt cataactcaca cttcatc 27

<210> 28
 <211> 30
 <212> DNA
 <213> Synthetic Primer
 <400> 28
 cggaattctc acacttcac ctcctatctc 30

<210> 29
 <211> 32
 <212> DNA
 <213> Synthetic Primers
 <400> 29
 gctctagaga tcagattgat gttataggaa tg 32

<210> 30
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INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 01/01775

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61L9/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61L C09B C11D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, FSTA, INSPEC, COMPENDEX, CHEM ABS Data, EMBASE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 780 527 A (O'LEARY NICHOLAS) 14 July 1998 (1998-07-14) cited in the application column 1, line 48 - line 51 column 2, line 66 - column 3, line 38 ---	1-13
A	US 4 362 841 A (MINATONO SHOBU ET AL) 7 December 1982 (1982-12-07) column 4, line 11 - line 18 column 4, line 56 - column 5, line 6 column 5, line 32 - line 39 ---	1-13
A	US 5 419 879 A (VLAHAKIS EFTICHIOS V ET AL) 30 May 1995 (1995-05-30) column 3, line 29 - line 39 example 1 --- -/--	1-13

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

Z document member of the same patent family

Date of the actual completion of the international search

17 August 2001

Date of mailing of the international search report

24/08/2001

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